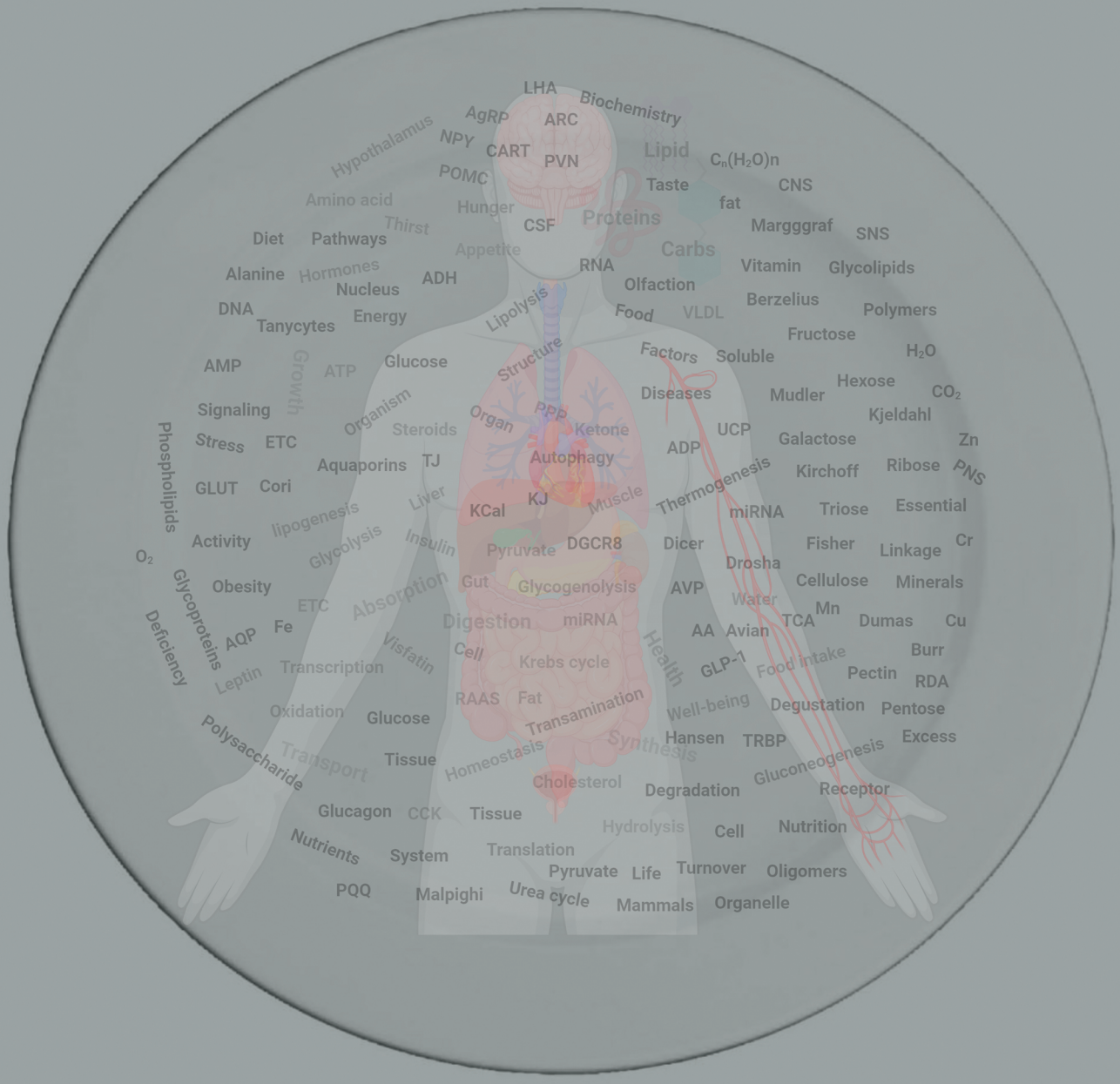
# NUTRITIONAL BIOCHEMISTRY: FROM THE CLASSROOM TO THE RESEARCH BENCH




Sami Dridi

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# Nutritional Biochemistry: From the Classroom to the Research Bench

Authored by

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# Nutritional Biochemistry: From the Classroom to the Research Bench

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# PREFACE

The idea of writing a book has had a relatively long gestation period. From a young age in the mid-eighties, as a biology and biochemistry volunteer tutor for underrepresented and disadvantaged high school students in Tunisia, I have had a profound desire to contribute to the personal and professional development of others. I feel the most fulfilled when I am educating and serving others. At that time, several students suggested that my notes could be organized and compiled in a text book. Unfortunately, although the idea was inspiring, I couldn't write the book for diverse reasons including lack of infrastructure (computer, etc.) and scientific maturity as well as economic disadvantages and constraints.

As I am drawn to the challenges of teaching and research because it gives me the opportunity to mentor students and contribute to their intellectual growth, which I consider to be the most valuable and worthwhile accomplishment, I joined the University of Arkansas in 2013 to conduct research on avian molecular nutrition and to develop and taught a biochemical nutrition course (POSC/ANSC 5143). At the beginning of this class, my students from different departments (poultry, animal, and food science, kinesiology, and nursing) and I used several biochemistry and nutrition conventional textbooks that we ordered through textbook department. After interaction with several students, the idea of organizing and collate my course notes in a text book was revived and regenerated. Taking advantage of technology expanding and advancement, I decided to compile my course in an electronic book (book) to enhance the learning process, encourage the students' creativity and learning autonomy, and reduce the burden of carrying heavy textbooks. This in turn, allows me to gain new perspectives on biochemical nutrition topics that inform my research, and reexamine the key ideas and assumptions that shape the production of knowledge in my field.

This book is intended to provide the readers with a comprehensive account of the interrelationship of nutrition and metabolism as well as an understanding of physiological changes that occur in the whole body as a result of excessive or deficient diets, and the endocrine and molecular regulatory mechanisms controlling such changes.

I tried to keep the book current with latest scientific advances and at the same time maintain a clear and readable style. It is my wish that this book will be revised and updated every five years because of the plenitude of new information and progress. Mrs. Humaira Hashmi, In-charge eBook department, at Bentham Science has been a source of support and encouragement. I owe a debt of gratitude to my family, my wife and my kids, for their unflagging support and their tolerance of my absence. Also, my students were the original inspiration for this book and I remain endlessly grateful to them, because from them I learn how to think and how to communicate knowledge in the most efficient, clear, and meaningful way.

## CONSENT FOR PUBLICATION

Not Applicable.

## CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENT

Declared none.

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# INTRODUCTION

Biochemical nutrition can be defined as the science of nutrition and chemical basis of life. It is a combination of two very broad, but tightly interconnected and complementary disciplines. As the cell is the basic unit of life, and nutrition is meant to provide the necessary energy for cellular functions (maintenance, proliferation, differentiation, division, etc.), thus biochemical nutrition can also be described as the study of the interaction between nutrients and the chemical constituents of living cells and of the reactions and processes they undergo. Following this definition, biochemical nutrition encompasses various and diverse aspects of biochemistry (the study of chemical characteristics and reactions of a particular living organism or biological substance), nutrition (the study of nutritional needs of a particular living organism), dietetics (diet formulation), integrative physiology (the study of body function), pathology (the study of causes and effects of diseases), cellular biology (the study of cell structure and function), molecular biology (the study of the structure and function of cellular macromolecules such as proteins and nucleic acids), neuroendocrinology (the study of the physiological interactions between the central nervous system and the endocrine system), immunology (the study of the immune system), microbiology (the study of microorganisms and their interaction with the host), behavior (the study of organismal action and response patterns to stimuli or stress), and more recently modeling and computational biology (the study of biological system networks using algorithms and models). The ultimate goal of biochemical nutrition is to help apprehend the origin of life by unraveling, at the molecular levels, all the chemical pathways associated with living cells, to define their energetic (nutrient) requirements, and to integrate biochemical and nutritional knowledge into efforts to not only maintain life and well-being through health improvement, but also to understand diseases for subsequent development of effective preventions or therapies.

The major objectives of the present book are to provide students and readers with a detailed, simplified, and comprehensive account of the interrelationship of nutrition and metabolism which is defined as the totality of chemical processes that occur in a living organism in order to maintain life, an understanding of physiological changes that occur in specific organs and in the whole body as a result of feeding diets with excess or deficient amounts of nutrients, and the endocrine and molecular regulatory mechanisms controlling such changes. As there are considerable differences in metabolism, nutrient requirement, and sometime in molecular pathways between mammalian and non-mammalian species, a comparative approach is often taken.

I know that biochemistry and nutrition present sometime an unusual challenge for students due to their diverse backgrounds, learning preferences, and aptitude and levels of interest. By writing this book, my fervent hope is to help diverse students from anywhere at any time to learn fast, integrate relevant principles in physiology, biochemistry, and molecular signaling pathways as they relate to nutrition of the whole organism, and to evaluate current nutrition concepts with a better understanding of how nutrition affects health, welfare, and performance. This book should enable students to formulate a biochemical approach to an experimental nutrition problem thus supporting the application of basic sciences and problem solving skills.

After an overview of the need of food and water (chapter 1), and describing the cell and organ system components (Chapter 2), the book focuses on the regulation of food intake from the factor influencing appetite to the central and peripheral underlying mechanisms (chapters 3, 4, and 5). In chapter 6, author will discuss water intake and water homeostasis regulation. In chapters 7, 8, and 9, protein, carbohydrate, and lipid metabolism are addressed from digestion and absorption to transport, utilization, synthesis, degradation, and molecular regulation. In conclusion, chapter 10 briefly summarizes the whole objective of the book.

# DEDICATION

To my beloved parents, may their souls be blessed in eternal peace

To my caring wife Stephanie and my kids Jalila and Noham

& to all who have inspired me –my children, family, students, mentors, and colleagues

# CHAPTER 1

# Setting the Stage: The Need of Water and Food

**Abstract:** This chapter sets the stage and provides current knowledge related to water and food necessities. Both water and food are essential for life and major keys to survival. Although water is not included in the diet formulation, it is considered an inorganic nutrient, and it is consumed mostly as drinking water and from feedstuffs. A further source of water is metabolic water or oxidation water, which is produced when macronutrients (carbohydrates, fats, and proteins) are oxidized to yield energy. Water comprises 75% body weight in infants to 55% in the elderly and is essential for cellular homeostasis. Similarly, the macronutrients provide energy (measured in Kcals) and essential components to sustain cellular homeostasis and life. These macronutrients are consumed in different combinations and ratios to help achieve different goals and health (disease) states. In this chapter, a brief description of these nutrients is provided.

**Keywords:** Food, Water, Nutrients, Macronutrients, Energy, Proteins, Carbohydrates, Fats.

## INTRODUCTION

Before discussing various aspects of biochemistry and metabolism, one might ask the following two basic questions: why do we need to eat? And why do we need to drink water? The simple and instinctive answers are: we eat because we are hungry and drink because we are thirsty. By eating food and drinking water, we live, we survive, and we allow our bodies to accomplish various tasks and physical works on a daily basis. An adult eats about a ton of food and drinks around 1095 L a year, and the human body contains about 60% water- a total of 42 L in a 70-kg person. It is like a power station, which requires fuel to generate energy and power the turbine. Food and water are the fuel that provides the body with the necessary **nutrients** and **energy (**metabolic fuels).

A brief description of nutrients and energy will be given for an introductory purpose, and they will be discussed in-depth in later chapters.

**Sami Dridi**

## 1.1. Nutrients

By definition, nutrients are substances used by an organism to survive, grow, and reproduce. Thus, they are building blocks of all organisms. They can be categorized into two types: macronutrients (or Major nutrients) and micronutrients. Macronutrients, which are consumed in great (gram, g) amounts, comprise carbohydrates, proteins, lipids or fats, and ethanol (alcohol). As these nutrients contain carbon-hydrogen bonds, they can also be categorized as organic nutrients. Micronutrients, however, are usually consumed in small (milligram, mg) quantities, include vitamins and minerals. Vitamins are organic, however minerals are inorganic nutrients. Although nutrients are dietary essentials, not all animals and all species require all nutrients. It has long been considered that all animals, with the exceptions of primates, humans, guinea pigs, and fish, can produce their own vitamin C or ascorbic acid [1]. Humans and primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, a rate-limiting enzyme in the biosynthesis of vitamin C through the glucuronic acid pathway [2]. Thus, vitamin C must be obtained through the diet, and an intake of 90-100 mg of vitamin C is required for nonsmoking men and women [3]. A deficient diet in vitamin C causes scurvy disease [4 - 7]. As a comparison, a typical 70 kg goat is capable of producing over 13 g of vitamin C daily [1]. Similarly, adult ruminant animals are capable of synthesizing B-complex vitamins (thiamin, riboflavin, niacin, biotin, folic acid, pyridoxine or B6, pantothenic acid, and B12) in their rumen flora and do not normally have a dietary requirement for it [8 - 10].

The ambiguity about what is and what is not a nutrient as well as for their specific requirements, still exists. For instance, glucose and other sugars are commonly considered to be nutrients however there are no specific requirements for individual sugar. Instead, there is a collective requirement for carbohydrates. Similarly, there is a combined requirement for fatty acids and proteins. Some of these individual sugars, fats, or amino acids can be omitted from the diet if appropriate dietary adjustments are made. For minerals and vitamins, the requirements are unambiguous because they have specific metabolic roles that cannot be replaced by other nutrients.

Following a myriad of biochemical processes during ingestion, digestion, metabolism, and storage throughout the organism, the purpose of food (nutrients) is to provide the required energy (metabolic fuels) for the body needs and thereby maintain the stability of its *milieu interieur* (internal environment). As evidenced from the homeostatic perspective of Claude Bernard and Walter Cannon [11, 12], the body is able to monitor its internal conditions and make the necessary adjustments to sustain its stability, referred to us as energy homeostasis or

homeostatic control of energy balance. Obviously, energy intake has to be appropriate for the level of energy expenditure, and neither excess intake nor a deficiency is desirable. In fact, an imbalance between energy inflow and outflow that results from gene-environmental interactions can derive a positive (body weight gain) or negative (body weight loss) energy balance. Total energy expenditure is composed primarily of basal metabolic rate (also known as resting energy expenditure or resting metabolic rate), diet-induced thermogenesis (also called specific dynamic action, the specific effect of food, or thermic effect of food), exercise or physical activity, and adaptive thermogenesis. Each of these components will be discussed in detail in later chapters. As the energy used in various activities can be measured, as can the metabolic energy yield of the foods that provide the fuel for that work, it is possible to calculate the balance between the energy intake and energy expenditure.

### *1.1.1. Macronutrients*

#### *1.1.1.1. Proteins*

Protein was first discovered by the Dutch chemist Gerhardus Johannes Mulder in 1837, who described it as a nitrogen-containing part of food essential to life. One year later, Jons Jacob Berzelius supported the theory of Mulder and proposed the name "protein" which is derived from the Greek word "*proteos*", and means "primary" or "first, for most" because it appears to be the primitive or principal substance of animal nutrition. In addition to carbon, oxygen, and hydrogen, protein also contains nitrogen and sulfur [13].

Proteins are essential parts of the diet (Table **1.1**) and they are composed of amino acids. Plant and animal proteins are composed of about 20 amino acids, organized in various sequences to form specific proteins. Intriguingly, there is a great number (over 900) in plants that are non-protein amino acids with no role in animal nutrition [14]. During digestion, proteins are broken down in the digestive tract to free amino acids that, after absorption, are used to exert significant biological functions and are also used by the body to rebuild new proteins and other necessary molecules such as neurotransmitters and hormones. The human body, for instance, can make some amino acids (non-essential or dispensable amino acids), but others must be obtained from the diet; these are so-called essential or indispensable amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) [15]. In addition to the abovementioned essential amino acids, poultry and swine need arginine [16].

**Table 1.1. Food composition charts. Macronutrients are in g/100g, minerals and vitamins are in mg/100g, and E in kcal. Carbs, carbohydrates; E, energy; - traces.**

| - | Food | Protein | Carbs | Fat | Thiamin | Na | E |
|---|---|---|---|---|---|---|---|
| - | Flounder, baked | 25 | 0 | 11 | 0.06 | 235 | 200 |
| **Fish** | Haddock, fried | 21 | 4 | 8 | 0 | 180 | 175 |
| - | Lobster, boiled | 22 | 0 | 3 | 0.08 | 330 | 120 |
| - | Fillet steak, fried | 28 | 0 | 9 | 0.08 | 85 | 200 |
| - | Sirloin, roast | 24 | 0 | 21 | 0.06 | 55 | 285 |
| **Meat** | Chicken, boiled | 29 | 0 | 7 | 0.06 | 80 | 185 |
| - | Duck, roast | 20 | 0 | 29 | 0 | 80 | 340 |
| - | Pork, grilled | 22 | 0 | 19 | 0.5 | 70 | 260 |
| - | Turkey, roast | 28 | 0 | 7 | 0 | 50 | 170 |
| - | Veal, roast | 31 | 4 | 8 | 0 | 110 | 215 |
| - | Cheese, camembert | 23 | - | 23 | 0.05 | 1410 | 300 |
| - | Danish blue | 23 | - | 29 | 0.03 | 1420 | 355 |
| **Milk products** | Swiss cheese | 29 | - | 29 | 0.01 | 155 | 375 |
| - | Milkshake, flavored | 3 | 17 | 5 | 0.04 | - | 120 |
| - | Yoghurt, favored | 3 | 11 | 4 | 0.04 | 40 | 100 |
| - | Cow milk, skim | 10 | 60 | - | 0.1 | 180 | 265 |
| - | Apple | 0.2 | 9 | - | 0.03 | 2 | 35 |
| **Fruits** | Apricot | 0.5 | 6 | - | 0.04 | - | 25 |
| - | Raspberries | 0.9 | 6 | - | 0.02 | 3 | 25 |
| - | Watermelon | 0.2 | 3 | - | 0.01 | 2 | 10 |
| **Egg** | Egg, boiled | 12 | - | 11 | 0.08 | 140 | 145 |
| - | Egg, fried | 14 | - | 20 | 0.07 | 220 | 230 |
| - | Barely, boiled | 3 | 28 | 1 | - | 1 | 120 |
| - | Wheat, starch-red | 45 | 37 | 8 | 0.2 | 610 | 390 |
| - | Bread, wheat | 14 | 48 | 2 | 0.2 | 540 | 205 |
| **Cereal products** | Cake, fruit | 5 | 58 | 13 | 0.08 | 250 | 355 |
| - | Pasta, boiled | 4 | 25 | 1 | 0.01 | 8 | 115 |
| - | Pizza, cheese | 9 | 25 | 12 | 0.1 | 340 | 235 |
| - | Rice, boiled | 2 | 30 | - | 0.01 | 2 | 125 |
| - | Lentils, boiled | 8 | 17 | 1 | 0.1 | 10 | 100 |
| - | Asparagus, boiled | 2 | 1 | - | 0.05 | 1 | 9 |
| **Vegetables** | Soya, boiled | 11 | 11 | 6 | 0.2 | 2 | 130 |

*(Table 1) cont.....*

| - | Food | Protein | Carbs | Fat | Thiamin | Na | E |
|---|---|---|---|---|---|---|---|
| - | Chickpeas, cooked | 8 | 22 | 3 | 0.1 | 850 | 145 |
| **Nuts** | Almonds | 17 | 4 | 54 | 0.2 | 6 | 555 |
| - | Peanuts, raw | 24 | 6 | 34 | 0.6 | 4 | 395 |
| **Fat** | Butter, salted | 0.4 | - | 81 | - | 840 | 730 |
| - | Vegetable oil | - | 0 | 100 | - | - | 900 |
| - | Beer | 0.3 | 2 | - | - | 9 | 40 |
| **Beverages** | Cola-type drink | - | 11 | 0 | 0 | 8 | 40 |
| - | Lemonade | - | 10 | 0 | - | 7 | 40 |

The values of the above food composition charts were obtained from http://apjcn.nhri.org.tw

As protein and amino acids contain nitrogen, the protein digestibility by livestock is often determined by measuring the nitrogen content of feed and feces, with the difference reflecting the amount of protein (amino acids) absorbed. In general, proteins contain about 16% of nitrogen (6.26g of protein contains 1 g nitrogen). The nitrogen (N) content is measured by Kjeldahl[1] procedure, and the crude proteins (CP) are determined using the following equation: CP= N x 6.25.

### *1.1.1.2. Carbohydrates*

The name of carbohydrate was originally assigned to substances thought to be hydrates of carbon and having the formula $C_n(H_2O)_n$ where not only the molar ratio of carbon to hydrogen to oxygen is 1:2:1, but also the ratio of carbon to water is 1:1. Therefore, carbohydrate is literally means “carbon with water or water of carbon”. In 1747, the German chemist Andreas Sigismund Marggraf discovered beet sugar. In 1811, the Russian chemist Constantine Kirchoff isolated crystalline sugar from sweet syrup obtained from starch under the action of acids. In 1844, Carl Schmidt designated carbohydrates (kohlenhydrate) as compounds containing carbon, hydrogen, and oxygen and showed that sugar was also found in the blood. In 1838, the French chemist Jean Baptiste Andre Dumas named the molecule glucose. The structure of simple sugars, including glucose, was established by about 1900, mainly by the brilliant work of the Germain chemist Emil Fisher who thereby laid the foundations of carbohydrate chemistry. The main members of the complex carbohydrate macromolecule are plant starch, pectin, cellulose, and gums. Simple carbohydrates encompass hexose monosaccharides (glucose, galactose, and fructose) and the disaccharide maltose (glucose-glucose), sucrose (glucose-fructose), and lactose (glucose-galactose). Other carbohydrates include trioses (glycerose), tetroses (erythrose), and pentoses (ribose and desoxyribose), which are important constituents of nucleic acids. Today, carbohydrates comprise polyhydroxy aldehydes, ketones, alcohols, acids,

and amines, their simple derivatives and the products formed by the condensation of these different compounds through glycosidic linkages (mainly oxygen bridges) into oligomers (oligosaccharides which yield three to ten monosaccharides on hydrolysis) and polymers (polysaccharides which yield more than ten oligosaccharides). Carbohydrates can also link proteins or lipids to form glycoproteins or glycolipids, respectively [17].

Carbohydrates are the major dietary energy source for most animals, with the exception of carnivores. Plant tissues contain pigments including chlorophyll and carotenoid that harness solar energy to provide electrons and produce carbohydrates according to the following reaction: Solar energy + $6CO_2$ + $6H_2O$ → $C_6H_{12}O_6$ + $6O_2$. According to rough estimates, more than 100 billion tons of carbohydrates are formed each year on the earth from carbon dioxide and water by the photosynthesis process. When animals digest plants, the energy contained in carbohydrates is converted into another form of energy that can be utilized by living cells and organisms (See chapter 6, section 6.3.2.4).

### *1.1.1.3. Lipids*

Lipids are organic compounds of plant and animal tissues that are oily (fatty acids or their derivatives) and are insoluble in water but soluble in organic solvents like ether, acetone, and chloroform. The lipid content in feeds is determined by diethyl ether extraction and is often referred to as the ether extract. In the early 1900s, dietary fat was viewed simply as energy-rich sources interchangeable with carbohydrates, having about 218% of the energy content of carbohydrates on an equal weight basis. In 1929, the Arkansian biochemist George Oswald Burr and his wife Mildred Burr challenged the above well-established view by demonstrating that free-fat diet caused the deficiency disease in rats and concluded that fat was an essential dietary component [18]. Their discovery of essential fatty acids (linoleic and linolenic acids) was a paradigm-changing finding and it is now viewed as one of the milestone discoveries in lipid research. In 1933, Arild Hansen (Burr's student) found infant eczema to respond to supplement of lard which contained both linoleic and arachidonic acids [19]. In 1938, arachidonic acid was determined to be an essential fatty acid.

Fat and lipids vary considerably in size and polarity, ranging from hydrophobic triglycerides and sterol esters to more water-soluble phospholipids and cardiolipins. They also differ in the number of carbon atoms and in the amount of hydrogen they contain. For example, those which are fully saturated with hydrogen are named saturated fatty acids; however, the unsaturated fatty acids incorporate one or more carbon-carbon double bonds that are not saturated with hydrogen. Dietary lipids also include cholesterol and phytosterols. Unlike other

macronutrients, and due to non-water miscibility, lipids undergo different processing during digestion, absorption, transport, storage, and utilization (see chapter 7, sections 7.2.).

### *1.1.2. Micronutrients*

#### *1.1.2.1. Minerals*

Mineral elements are the inorganic constituents of plant and animal tissues. In animal nutrition, they are categorized into two classes:

1. Macro-minerals which refer to those elements needed by the body in milligram quantities on a daily basis including sodium, potassium, chloride, calcium, phosphorus, and magnesium. They serve as electrolytes and they have a structural as well as metabolic regulation function.
2. Micro-minerals are the elements needed by the body in far smaller amounts. They are divided in two groups: trace-minerals which include iron (Fe), copper (Cu), and zinc (Zn) while the other group, ultra-trace minerals, contains chromium (Cr), manganese (Mn), fluorine (F), iodine (I), cobalt (Co), selenium (Se), silicon (Si), arsenic (As), boron (B), vanadium (V), nickel (Ni), cadmium (Cd), lithium (Li), lead (Pb), and molybdenum (Mo). Of these trace-minerals, only zinc, iron, iodine, and selenium have a recommended dietary allowance (RDA) (Table **1.2**) because they are the most studied. For some of the other minerals there is an intake recommendation known as generally recognized as safe and adequate (GRSA) (Table **1.3**). No intake recommendation, however, has been made for the remaining minerals, including cobalt which is important for microbial synthesis of vitamin $B_{12}$.

**Table 1.2. Recommended dietary allowance (RDA) for minerals.**

| - | RDA | | | | |
|---|---|---|---|---|---|
| **Group** | **Age** | **Fe (mg)** | **Zn (mg)** | **I (mg)** | **Se (µg)** |
| **Infants** | 0-6 months | 6 | 5 | 40 | 10 |
| - | 7-12 months | 10 | 5 | 50 | 15 |
| - | 1-3 years | 10 | 10 | 70 | 20 |
| **Children** | 4-7 years | 10 | 10 | 90 | **20** |
| - | 8-11 years | 10 | 10 | 120 | 30 |
| - | 12-14 years | 12 | 15 | 150 | 40 |
| **Males** | 15-18 years | 12 | 15 | 150 | 50 |

*(Table 2) cont.....*

| - | RDA | | | | |
|---|---|---|---|---|---|
| **Group** | **Age** | **Fe (mg)** | **Zn (mg)** | **I (mg)** | **Se (µg)** |
| - | 19-24 years | 10 | 15 | 150 | 70 |
| - | 25-50 years | 10 | 15 | 150 | 70 |
| - | +51 years | 10 | 15 | 150 | 70 |
| - | 12-14 years | 15 | 12 | 150 | 45 |
| - | 15-18 years | 15 | 12 | 150 | 50 |
| **Females** | 19-24 years | 15 | 12 | 150 | 55 |
| - | 25-50 years | 15 | 12 | 150 | 55 |
| - | +51 years | 10 | 12 | 150 | 55 |
| **Pregnancy** | | 30 | 15 | 175 | 65 |
| **Lactation** | 0-6 months | 15 | 19 | 200 | 75 |
| - | 7-12 months | 15 | 16 | 200 | 75 |

Fe, Iron; I, Iodide; Se, Selenium; Zn, Zinc.

**Table 1.3. Safe intake for selected minerals.**

| Minerals | Safe Intake/day |
|---|---|
| **Cu** | 1.5-3.0 mg |
| **F** | 1.4-4.0 mg |
| **Mn** | 2-5.0 mg |
| **Cr** | 50-200 µg |
| **Mo** | 75-250 µg |

Cr, Chromium; Cu, Copper; F, Fluoride; Mn, Manganese; Mo, Molybdenum

Although these elements play a pivotal role, inadvertent exposure to a variety of minerals can elicit a toxic response (Table **1.4**). Similarly, deficiency can lead to pathology and diseases (Table **1.4**).

**Table 1.4. Health conditions related to some of micro-mineral deficiency or excess.**

| Mineral | Deficiency | Excess-Toxicity |
|---|---|---|
| **Cu** | Myeloneuropathy [20], myelodysplasia [21], Osteoporosis, osteoarthritis, colon cancer, cardiovascular disease [22 - 25] | Alzheimer [26]<br>Cirrhosis [27]<br>Anemia, leukopenia [28],<br>Tachycardia [29], Wilson disease [30]<br>Menkes disease [31]<br>Idiopathic Cu toxicosis |
| **Fe** | Anemia [32] | Hemochromatosis |

*(Table 4) cont.....*

| Mineral | Deficiency | Excess-Toxicity |
|---|---|---|
| **Zn** | Acne, eczema, xerosis, alopecia, stomatitis, angular cheilitis, burning mouth syndrome, and night blindness [33, 34] | Abdominal pain, nausea, vomiting, diarrhea, red blood cell microcytosis, neutropenia, |
| **I** | Goitre, Cretinism, thyroiditis [35, 36] | Thyroid dysfunction |
| **Co** | Pernicious anemia, neurological disorders Wool break (sheep) and scours in calves | Cardiomyopathy, goiter, kidney and nerve damage |
| **F** | Dental caries, osteoporosis, bone disorder [37 - 39] | Skeletal fluorosis, bone fractures, irritable-bowel syndrome, arthritis, ankylosing spondylitis, nephrotoxicity [40, 41] |
| **Mn** | Skeletal deformation, inhibit collagen production, Perthes' disease, arthritis [42 - 44] | Toxicity, liver failure, brain damage [45, 46] |
| **Se** | Keshan disease, Kashin-Beck disease, hypothyroidism, goiter, cretinism, recurrent miscarriage [47] | Dyspnea, respiratory failure, endocarditis, myocarditis, tibia erosion, chronic selenosis |

Co, Cobalt; Cu, Copper; F, Fluorine; Fe, iron; I, Iodine; Mn, Manganese; Se, selenium; Zn, Zinc

### *1.1.2.2. Vitamins*

Perhaps the earliest articulation of the "vitamin theory" came from Jean Baptist Dumas (French chemist, 1800-1884), Frederick Gowland Hopkins (English biochemist, 1861-1947), and Nicolai Ivanovich Lunin (Soviet pediatrician, 1853-1937), who showed that in addition to proteins, fats, carbohydrates, salts, and water, certain special substances (named accessory factors and later called vitamin" are also needed for the animal to develop and live normally [48 - 51]. In 1912, the Polish biochemist Casimir Funk proposed the term "vitamine or vital amine" instead of accessory food factors because these amines were vital to the animal survival [52]. Later, after it discovered that not all vitamins contained amines, the final "e" vowel was removed from the word.

Vitamins are a large group of potent organic compounds other than proteins, carbohydrates, and lipids that have specific roles in metabolism and are required in the diet in minute amounts. They are divided into two categories based on their solubility characteristics. The fat-soluble vitamins contain vitamin A (retinol), vitamin D (cholecalciferol), vitamin E (α-tocopherol), and vitamin K (phylloquinone), which are soluble in one or more solvents such as alcohol or chloroform [53]. The water-soluble vitamins, including vitamin C (ascorbic acid) and the members of the vitamin B-complex [vitamin B1 (thiamin), vitamin B2 (riboflavin), vitamin B6 (pyridoxine), vitamin B12 (cyanocobalamin), niacin (nicotinic acid), folacin (folic acid), biotin, and pantothenic acid] [54].

Deficiency in one or more vitamins causes a specific disease, which is cured or prevented only by restoring the vitamin to the diet. Similarly, a toxic condition can be developed when high levels of the vitamin are consumed. As an example, vitamin A deficiency can result in blindness [55 - 57] and hypervitaminosis A can lead to intoxication in humans, rodents, and chickens [58 - 61]. In chickens, a depressed growth rate and an encrustation of the eyelids were observed. In rats, the obvious clinical signs were bone fractures. In humans, hypervitaminosis A is characterized by increased intracranial pressure resulting in headaches, blurring of vision, skin lesions, anorexia, nausea, vomiting, and weight loss.

#### *1.1.2.3. Other Organic Nutrients*

Choline, carnitine, inositol, and several other biological active compounds such as pyrroloquinoline quinone (PQQ), ubiquinone, lipoic acid, bioflavonoids, and pseudovitamins are not actually considered minerals or vitamins, but they are known to be important nutrients needed for many functions of the body. The structure, metabolism and function of these nutrients will not be discussed in this edition, and I hope to include it in detail along with micro-nutrients in the next edition.

### *1.1.3. Water*

Although water is not included in the diet formulation in livestock or domestic animals, it is considered an inorganic nutrient as it does not possess carbon-hydrogen bonds. Water is generally required in greater quantity than any other orally ingested substance, and it is consumed mostly as drinking water. In addition to beverages, feedstuffs can provide 22% of total water intake and up to 60-90% if they are fruits or vegetables. A further source of water is metabolic water, also known as oxidation water, which is produced when macro-nutrients (carbohydrates, fats, and proteins) are oxidized to yield energy. This accounts for about 12% of total water intake and more on a high fat diet, or when metabolizing fat reserves. Indeed, animal metabolism produces about 100, 42, or 60g of metabolic water per 100g of fat, proteins, or carbohydrates, respectively [62].

As for nutrients, water homeostasis is a balance between water intake and water outputs. As shown in Table **1.5**, in adult men, urine accounts for 47% of the total fluid output from the body. The remainder is made up of sweat (22%) produced by the sweat gland, water in exhaled air (11%), insensible losses *via* the skin (17%), and a relatively small amount (about 3%) in feces. Although water losses in exhaled air and other insensible losses are relatively constant, sweat losses depend largely on the surrounding temperature and physical activity. Water losses

in feces depend on the diet; the more the diet is rich in fibers more the water losses are, because the fiber retains water in the intestinal tract and thereby softening the feces.

**Table 1.5. Daily fluid balance in human.**

| - | Gender<br>Adult Man Adult Woman | | | | |
|---|---|---|---|---|---|
| **-** | **-** | **mL/day** | **% of total** | **mL/day** | **% of total** |
| - | Fluids | 1950 | 65 | 1400 | 67 |
| **Input** | Water in food | 700 | 23 | 450 | 21 |
| - | Metabolic water | 350 | 12 | 250 | 12 |
| - | **Total** | **3000** | **100** | **2100** | **100** |
| - | Urine | 1400 | 47 | 1000 | 48 |
| - | Sweat | 650 | 22 | 420 | 20 |
| **Output** | Exhaled air | 320 | 11 | 320 | 15 |
| - | Insensible losses | 530 | 17 | 270 | 13 |
| - | Water in faeces | 100 | 3 | 90 | 4 |
| - | **Total** | **3000** | **100** | **2100** | 100 |

The question is whether drinking water alone is sufficient to balance large losses in sweat after intense exercise or under high environmental temperatures. The answer is probably not because sweating involves not only water losses but also mineral salts losses. Milk, fruit juices, and various sports drinks contain balanced mixtures of mineral salts in the same proportion as they are lost in sweat.

Several species, including desert animals (*e.g.* pack rat, kangaroo rat) survive on metabolic water. Because of lack of sweat gland, high concentrated urine excretion, and low evaporation rate from the expired air, the kangaroo rat has a very low rate of water loss [63]. The camel is able to survive for a considerable time in desert conditions without drinking because it metabolizes the fat reserve stored in its hump [64]. Marine mammals such as seals, sea lions, walrus, and whales and most marine fish, however, obtain their water from their food [65].

As shown in Table **1.6**, there is a large difference in water requirements between species. One of the factors that influence this difference is the nature of the nitrogenous end products of protein metabolism excreted in the urine.

**Table 1.6. Estimated average water consumption of various species in a temperate climate (adapted from NRC 1994).**

| Species | Species | Water (L/day) |
|---|---|---|
| *Homo Sapiens* | *Man* | ~3 |
| *Homo Sapiens* | *Woman* | ~2.1 |
| *Bos Taurus* | *Beef cattle* | 26-66 |
| *Bos Taurus* | *Dairy cattle* | 38-110 |
| *Equus Ferus Caballus* | *Horse* | 30-45 |
| *Sus scrofa domesticus* | *Pig* | 11-19 |
| *Ovis aries* | *Sheep* | 4-15 |
| *Capra hircus* | *Goat* | 4-15 |
| *Gallus gallus domesticus* | *Chicken* | 0.2-0.4 |
| *Meleagris gallopavo* | *Turkey* | ~0.4 |

In fact, large mammals require a large amount of water to dilute urea which is toxic to the tissues unless in dilute solution. Birds excrete uric acid in a nearly solid form and therefore require less water than mammals. Fish excrete ammonia directly from the gills. The surrounding environmental temperatures, diet composition, feeding strategies, and the nature of the digestive tract influence water requirements. For instance, high protein diets in mammals increase the amount of water required to dilute urinary urea. Compared to non-ruminant species, ruminants require a larger amount of water to form a suspension of ingesta in the rumen. Feedstuffs with high water-absorbing characteristics such as dry hay augment the water requirements. Although water requirement is expected to increase during cold weather due to augmented-feed intake, it is more intensified under hot climates, due to the complex interplay between the hunger/satiety and thirst centers.

## 1.2. *Units of Energy*

Thousands of years ago, an inherent internal energy flow within the human body was discovered and named Qi by the Chinese and Prana by the Indians. According to traditional Chinese and Indian Medicines, this flowing energy regulates the human body functions. In 1779, the French chemist Antoine-Laurent Lavoisier coined the name Oxygen for the element released by mercury oxide and found that oxygen was essential for combustion and respiration, confirming his new fundamental law of nature "law of conservation of mass". In collaboration with the French mathematician Pierre-Simon Laplace, Lavoisier developed the caloric theory of heat by demonstrating that the expiration of carbon dioxide by mammals increased with physical activities. The oxidation of sugars and fats accounted for the energy needed for animal heat production [66]. Later and during the period 1803-1873, the German organic chemist Justus Freiherr von Liebig

asserted that protein was the only true nutrient serving as the source of energy for muscular contraction by the breakdown that was followed by the synthesis and then the excretion of urea.

Although Lavoisier named the calorimeter (calorimètre) by 1789, the word calorie was being used as unit of heat by 1824 [67]. It was defined as the amount of heat needed to raise the temperature of 1 g of water by 1°C. The calorie is still used to some extent in nutrition. In biological systems, however, the kilocalorie (kcal or $10^3$ cal also written as Calorie with a capital C) is used and is defined as the quantity of heat required to raise the temperature of 1 kg of water by 1°C. In collaboration with Lord Kelvin to develop the absolute scale of temperature (Kelvin scale), James Prescott Joule (English physicist and mathematician, 1818-1889) estimated the mechanical equivalent of heat as 4.1868 joules per calorie of work to raise the temperature of 1g of water by 1 Kelvin [68]. In biological systems, the kilojoule (kJ= $10^3$J) and Megajoule (MJ=$10^6$J) are used. The equation below are given to convert between calories and joules:

$$1 \text{ kcal} = 4.186 \text{ kJ and } 1 \text{ kJ} = 0.239 \text{ kcal.}$$

Justus Freiherr von Liebig was the first to suggest that animals have the capability to synthesize fats from sugars and starch. Other researchers built upon his work, confirming the abilities of animals to synthesize molecules from dietary metabolic fuels or metabolic energy (Table **1.7**).

**Table 1.7. Average energy yield of metabolic fuels.**

| - | Kcal/g | kJ/g |
|---|---|---|
| Carbohydrates | 4 | 17 |
| Protein | 4 | 16 |
| Fat | 9 | 37 |
| Alcohol | 7 | 29 |

Note that 1 kcal = 4.186 kJ and 1 kJ = 0.239 kcal

The metabolism of these fuels results in the production of carbon dioxide and water (and also urea in the case of proteins). They can be converted to the same end products chemically by burning in air. Although the metabolic pathways and processes in the body are complex, it is a fundamental law of chemistry that if the starting material and end products are the same (law of mass conservation), the energy yield is the same regardless of the route taken. Thus, the energy yield of foodstuffs can be determined by measuring the heat produced when they are burnt in the air.

Every living organism must capture, transduce, store, and use energy to live. Following the first Law of thermodynamics, this energy is conserved; though it can be changed from one form to another, it can be neither created nor destroyed. As a living organism is an open system, it is able to exchange both matter and energy with its surrounding environment and increase the entropy[2] of the universe and thereby follow the Second Law of thermodynamics. For instance, human or animals digest food by breaking it down into metabolic fuels (sugar, fatty acids, amino acids) and absorb them to build up cells and tissues and provide the energy necessary for the daily needs of the body. Although this process increase the body entropy, it decreases the order of the universe because the body dissipate (loses) energy *via* conduction, convection, and radiation as well as eliminating waste.

As the cell is the basic unit and the microcosm of life, next chapter will describe the components of cells.

## CONCLUSION

Each of water and food, including carbohydrates, proteins, fats, minerals, and vitamins, has a unique set of properties that influence health. Over the past century, there has been tremendous progress in defining the mechanisms by which the intake of each is regulated and the pathways by which each may contribute to energy homeostasis. This chapter aims to describe water and food and set the stage for the next chapters where their regulation and their physiological effects will be discussed in more detail.

## NOTES

[1] Kjeldahl method or Kjeldahl digestion, developed by Johan Kjeldahl, is a procedure for the quantitative determination of nitrogen contained in organic substances plus the nitrogen contained in the inorganic compounds ammonia and ammonium

[2] Entropy is a scientific concept, as well as a measurable physical property that is most commonly associated with a state of disorder.

## REFERENCES

[1] Chatterjee IB, Majumder AK, Nandi BK, Subramanian N. Synthesis and some major functions of vitamin C in animals. Ann N Y Acad Sci 1975; 258(1 Second Confer): 24-47. [http://dx.doi.org/10.1111/j.1749-6632.1975.tb29266.x] [PMID: 1106297]

[2] Flier JS, Underhill LH, Levine M. New concepts in the biology and biochemistry of ascorbic acid. N Engl J Med 1986; 314(14): 892-902. [http://dx.doi.org/10.1056/NEJM198604033141407] [PMID: 3513016]

[3] Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant

and health effects in humans. Am J Clin Nutr 1999; 69(6): 1086-107.
[http://dx.doi.org/10.1093/ajcn/69.6.1086] [PMID: 10357726]

[4] Gerster H. Human vitamin C requirements. Z Ernährungswiss 1987; 26(2): 125-37.
[http://dx.doi.org/10.1007/BF02019608] [PMID: 3307183]

[5] Desenclos JC. Relief food and vitamin C deficiency. Lancet 1987; 330(8556): 462-3.
[http://dx.doi.org/10.1016/S0140-6736(87)91006-3] [PMID: 2887771]

[6] Sato PH, Roth LA, Walton DM. Treatment of a metabolic disease, scurvy, by administration of the missing enzyme. Biochem Med Metab Biol 1986; 35(1): 59-64.
[http://dx.doi.org/10.1016/0885-4505(86)90058-7] [PMID: 3778677]

[7] Granger M, Eck P. Dietary Vitamin C in Human Health. Adv Food Nutr Res 2018; 83: 281-310.
[http://dx.doi.org/10.1016/bs.afnr.2017.11.006] [PMID: 29477224]

[8] Poe SE, Mitchell GE Jr, Ely DG. Rumen development in lambs. 3. Microbial B-vitamin synthesis. J Anim Sci 1972; 34(5): 826-9.
[http://dx.doi.org/10.2527/jas1972.345826x] [PMID: 5022491]

[9] Cover S, Dilsaver EM, Hays RM. Variation Among Lamb Carcasses in the B Vitamin Content of Meat. Science 1947; 105(2727): 364-5.
[http://dx.doi.org/10.1126/science.105.2727.364] [PMID: 17800879]

[10] Seck M, Linton JAV, Allen MS, Castagnino DS, Chouinard PY, Girard CL. Apparent ruminal synthesis of B vitamins in lactating dairy cows fed diets with different forage-to-concentrate ratios. J Dairy Sci 2017; 100(3): 1914-22.
[http://dx.doi.org/10.3168/jds.2016-12111] [PMID: 28109593]

[11] Cooper SJ. From Claude Bernard to Walter Cannon. Emergence of the concept of homeostasis. Appetite 2008; 51(3): 419-27.
[http://dx.doi.org/10.1016/j.appet.2008.06.005] [PMID: 18634840]

[12] Jeanrenaud B, Cusin I, Rohner-Jeanrenaud F. [From Claude Bernard to the regulatory system between the hypothalamus and the periphery: implications for homeostasis of body weight and obesity]. C R Seances Soc Biol Fil 1998; 192(5): 829-41. [From Claude Bernard to the regulatory system between the hypothalamus and the periphery: implications for homeostasis of body weight and obesity].
[PMID: 9871796]

[13] Vickery HB. The origin of the word protein. Yale J Biol Med 1950; 22(5): 387-93.
[PMID: 15413335]

[14] Wink M. Special nitrogen metabolism. 1997.
[http://dx.doi.org/10.1016/B978-012214674-9/50013-8]

[15] Thier SO, Alpers DH. Disorders of intestinal transport of amino acids. Am J Dis Child 1969; 117(1): 13-23.
[PMID: 4883974]

[16] Meade RJ. Biological availability of amino acids. J Anim Sci 1972; 35(3): 713-23.
[http://dx.doi.org/10.2527/jas1972.353713x] [PMID: 4626785]

[17] Paulson JC. Glycoproteins: what are the sugar chains for? Trends Biochem Sci 1989; 14(7): 272-6.
[http://dx.doi.org/10.1016/0968-0004(89)90062-5] [PMID: 2672447]

[18] Burr GO, Burr MM. Nutrition classics from The Journal of Biological Chemistry 82:345-67, 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. Nutr Rev 1973; 31(8): 248-9.
[PMID: 4586201]

[19] Hansen AE, Haggard ME, Boelsche AN, Adam DJD, Wiese HF. Essential fatty acids in infant nutrition. III. Clinical manifestations of linoleic acid deficiency. J Nutr 1958; 66(4): 565-76.
[http://dx.doi.org/10.1093/jn/66.4.565] [PMID: 13621281]

[20] Spinazzi M, De Lazzari F, Tavolato B, Angelini C, Manara R, Armani M. Myelo-optico-neuropathy in copper deficiency occurring after partial gastrectomy. J Neurol 2007; 254(8): 1012-7. [http://dx.doi.org/10.1007/s00415-006-0479-2] [PMID: 17415508]

[21] Kumar N, Elliott MA, Hoyer JD, Harper CM Jr, Ahlskog JE, Phyliky RL. "Myelodysplasia," myeloneuropathy, and copper deficiency. Mayo Clin Proc 2005; 80(7): 943-6. [http://dx.doi.org/10.4065/80.7.943] [PMID: 16007901]

[22] Stern BR, Solioz M, Krewski D, *et al*. Copper and human health: biochemistry, genetics, and strategies for modeling dose-response relationships. J Toxicol Environ Health B Crit Rev 2007; 10(3): 157-222. [http://dx.doi.org/10.1080/10937400600755911] [PMID: 17454552]

[23] Danks DM. Copper deficiency in humans. Annu Rev Nutr 1988; 8(1): 235-57. [http://dx.doi.org/10.1146/annurev.nu.08.070188.001315] [PMID: 3060166]

[24] Klevay LM. The influence of copper and zinc on the occurrence of ischemic heart disease. J Environ Pathol Toxicol 1980; 4(2-3): 281-7. [PMID: 7007558]

[25] Strain JJ. Newer aspects of micronutrients in chronic disease: copper. Proc Nutr Soc 1994; 53(3): 583-98. [http://dx.doi.org/10.1079/PNS19940067] [PMID: 7886057]

[26] Loef M, Walach H. Copper and iron in Alzheimer's disease: a systematic review and its dietary implications. Br J Nutr 2012; 107(1): 7-19. [http://dx.doi.org/10.1017/S000711451100376X] [PMID: 21767446]

[27] O'Donohue J, Reid M, Varghese A, Portmann B, Williams R. A case of adult chronic copper self-intoxication resulting in cirrhosis. Eur J Med Res 1999; 4(6): 252. [PMID: 10383882]

[28] Lazarchick J. Update on anemia and neutropenia in copper deficiency. Curr Opin Hematol 2012; 19(1): 58-60. [http://dx.doi.org/10.1097/MOH.0b013e32834da9d2] [PMID: 22080848]

[29] Langley A, Dameron CT. Copper and anesthesia: clinical relevance and management of copper related disorders. Anesthesiol Res Pract 2013; 2013: 1-10. [http://dx.doi.org/10.1155/2013/750901] [PMID: 23762044]

[30] Scheinberg IH, Sternlieb I. Wilson disease and idiopathic copper toxicosis. Am J Clin Nutr 1996; 63(5): 842S-5S. [http://dx.doi.org/10.1093/ajcn/63.5.842] [PMID: 8615372]

[31] Kaler SG. Metabolic and molecular bases of Menkes disease and occipital horn syndrome. Pediatr Dev Pathol 1998; 1(1): 85-98. [http://dx.doi.org/10.1007/s100249900011] [PMID: 10463276]

[32] Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron-deficiency anemia. N Engl J Med 1993; 329(23): 1691-5. [http://dx.doi.org/10.1056/NEJM199312023292303] [PMID: 8179652]

[33] Michaëlsson G. Diet and Acne. Nutr Rev 1981; 39(2): 104-6. [http://dx.doi.org/10.1111/j.1753-4887.1981.tb06740.x] [PMID: 6451820]

[34] Gurvits GE, Tan A. Burning mouth syndrome. World J Gastroenterol 2013; 19(5): 665-72. [http://dx.doi.org/10.3748/wjg.v19.i5.665] [PMID: 23429751]

[35] Knudsen N, Laurberg P, Perrild H, Bülow I, Ovesen L, Jørgensen T. Risk factors for goiter and thyroid nodules. Thyroid 2002; 12(10): 879-88. [http://dx.doi.org/10.1089/105072502761016502] [PMID: 12487770]

[36] Rose NR, Bonita R, Burek CL. Iodine: an environmental trigger of thyroiditis. Autoimmun Rev 2002;

1(1-2): 97-103.
[http://dx.doi.org/10.1016/S1568-9972(01)00016-7] [PMID: 12849065]

[37] Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007; 369(9555): 51-9.
[http://dx.doi.org/10.1016/S0140-6736(07)60031-2] [PMID: 17208642]

[38] Kleerekoper M. The role of fluoride in the prevention of osteoporosis. Endocrinol Metab Clin North Am 1998; 27(2): 441-52.
[http://dx.doi.org/10.1016/S0889-8529(05)70015-3] [PMID: 9669148]

[39] Riggs BL, Hodgson SF, O'Fallon WM, *et al.* Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. N Engl J Med 1990; 322(12): 802-9.
[http://dx.doi.org/10.1056/NEJM199003223221203] [PMID: 2407957]

[40] Gupta R, Kumar AN, Bandhu S, Gupta S. Skeletal fluorosis mimicking seronegative arthritis. Scand J Rheumatol 2007; 36(2): 154-5.
[http://dx.doi.org/10.1080/03009740600759845] [PMID: 17476625]

[41] Gessner BD, Beller M, Middaugh JP, Whitford GM. Acute fluoride poisoning from a public water system. N Engl J Med 1994; 330(2): 95-9.
[http://dx.doi.org/10.1056/NEJM199401133300203] [PMID: 8259189]

[42] Cotzias GC, Papavasiliou PS, Hughes ER, Tang L, Borg DC. Slow turnover of manganese in active rheumatoid arthritis accelerated by prednisone. J Clin Invest 1968; 47(5): 992-1001.
[http://dx.doi.org/10.1172/JCI105814] [PMID: 4868949]

[43] Fincham JE, van Rensburg SJ, Marasas WF. Mseleni joint disease--a manganese deficiency? S Afr Med J 1981; 60(12): 445-7.
[PMID: 7280891]

[44] Hall AJ, Margetts BM, Barker DJP, *et al.* Low blood manganese levels in Liverpool children with Perthes' disease. Paediatr Perinat Epidemiol 1989; 3(2): 131-6.
[http://dx.doi.org/10.1111/j.1365-3016.1989.tb00505.x] [PMID: 2734231]

[45] Borchers A, Epstein SE, Gindiciosi B, Cartoceti A, Puschner B. Acute enteral manganese intoxication with hepatic failure due to ingestion of a joint supplement overdose. J Vet Diagn Invest 2014; 26(5): 658-63.
[http://dx.doi.org/10.1177/1040638714544316] [PMID: 25080444]

[46] Kawata F. Studies on monoamine metabolism in the rat brain with overdosage of manganese. Nippon Hoigaku Zasshi 1990; 44(2): 137-46.
[PMID: 2402098]

[47] Moreno-Reyes R, Suetens C, Mathieu F, *et al.* Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. N Engl J Med 1998; 339(16): 1112-20.
[http://dx.doi.org/10.1056/NEJM199810153391604] [PMID: 9770558]

[48] Hopkins FG. The analyst and the medical man. Analyst (Lond) 1906; 31(369): 385b.
[http://dx.doi.org/10.1039/an906310385b]

[49] Lunin N. Über die Bedeutung der anorganischen Salze für die Ernährung des Thieres. Z F Physiol Chem 1881; 5: 31.

[50] Dumas J B A. Note sur la constitution du lait et du sang. Le Monit Sci 3 ser 778(1871)

[51] Hopkins FG. Feeding experiments illustrating the importance of accessory factors in normal dietaries. J Physiol 1912; 44(5-6): 425-60.
[http://dx.doi.org/10.1113/jphysiol.1912.sp001524] [PMID: 16993143]

[52] Funk C. The etiology of the defi ciency diseases. Beri-beri, polyneuritis in birds, epidemic dropsy, scurvy, experimental scurvy in animals, infantile scurvy, ship beri-beri, pellagra. J State Med 1912; 20: 341.

[53] Unglaub WG, Goldsmith GA, Gibbens J. Recent advances in nutrition and metabolism. II. Review of

the literature on vitamins, 1952. AMA Arch Intern Med 1954; 94(4): 618-47.
[http://dx.doi.org/10.1001/archinte.1954.00250040110010] [PMID: 13196744]

[54] Brown GM, Reynolds JJ. Biogenesis of the Water-Soluble Vitamins. Annu Rev Biochem 1963; 32(1): 419-62.
[http://dx.doi.org/10.1146/annurev.bi.32.070163.002223] [PMID: 14144485]

[55] Martini S, Rizzello A, Corsini I, *et al.* Vitamin A Deficiency Due to Selective Eating as a Cause of Blindness in a High-Income Setting. Pediatrics 2018; 141 (Suppl. 5): S439-44.
[http://dx.doi.org/10.1542/peds.2016-2628] [PMID: 29610168]

[56] Dowling JE, Wald G. Vitamin a Deficiency and Night Blindness. Proc Natl Acad Sci USA 1958; 44(7): 648-61.
[http://dx.doi.org/10.1073/pnas.44.7.648] [PMID: 16590255]

[57] Dunlap K, Loken RD. Vitamin a for Color-Blindness. Science 1942; 95(2474): 554.
[http://dx.doi.org/10.1126/science.95.2474.554.a] [PMID: 17790871]

[58] Lind T, Öhman C, Calounova G, *et al.* Excessive dietary intake of vitamin A reduces skull bone thickness in mice. PLoS One 2017; 12(4): e0176217.
[http://dx.doi.org/10.1371/journal.pone.0176217] [PMID: 28426756]

[59] Rodahl K. Hypervitaminosis A and scurvy. Nature 1949; 164(4169): 531.
[http://dx.doi.org/10.1038/164531a0] [PMID: 18229143]

[60] Baker JR, Howell JM, Thompson JN. Hypervitaminosis A in the chick. Br J Exp Pathol 1967; 48(5): 507-12.
[PMID: 6075435]

[61] Rodahl K. Hypervitaminosis A in the rat. J Nutr 1950; 41(3): 399-421.
[http://dx.doi.org/10.1093/jn/41.3.399] [PMID: 15428909]

[62] Mellanby K. Metabolic water and desiccation. Nature 1942; 150(3792): 21.
[http://dx.doi.org/10.1038/150021a0]

[63] Tracy R, Walsberg G. Developmental and acclimatory contributions to water loss in a desert rodent: investigating the time course of adaptive change. J Comp Physiol B 2001; 171(8): 669-79.
[http://dx.doi.org/10.1007/s003600100218] [PMID: 11765976]

[64] Warda M, Prince A, Kim HK, *et al.* Proteomics of old world camelid (Camelus dromedarius): Better understanding the interplay between homeostasis and desert environment. J Adv Res 2014; 5(2): 219-42.
[http://dx.doi.org/10.1016/j.jare.2013.03.004] [PMID: 25685490]

[65] Ortiz RM. Osmoregulation in marine mammals. J Exp Biol 2001; 204(11): 1831-44.
[http://dx.doi.org/10.1242/jeb.204.11.1831] [PMID: 11441026]

[66] Levere TH. Dimensions of a scientist: lavoisier and the chemistry of life. Science 1985; 229(4715): 751.
[http://dx.doi.org/10.1126/science.229.4715.751.a] [PMID: 17841492]

[67] Hargrove JL. History of the calorie in nutrition. J Nutr 2006; 136(12): 2957-61.
[http://dx.doi.org/10.1093/jn/136.12.2957] [PMID: 17116702]

[68] Joule JP. On the calorific effects of magneto-electricity, and on the mechanical value of heat. Philos Mag 1843; 23: 263.

# CHAPTER 2

# The Cell: The Basic Functional Unit of Life

**Abstract:** Cell is the smallest and the basic functional unit of life. Every organism, whether prokaryotic, archaeans, or eukaryotic, is composed of a basic building block which is the Cell. A cell consists of a cell membrane, a nucleus, and a cytoplasm where intricate arrangements of fine fibers and organelles lie. Cells are specialized for a specific purpose, they form tissues which in turn form organs, and several organs make up the system, and several systems that function together form the organism. The present chapter aims to describe the body, tissues, system, organism, and the structure and function of the major cell organelles with a focus on eukaryotic cells.

**Keywords:** Cell, Organism, Organ, Organelle, Tissue, System.

## INTRODUCTION

Cell is the basic structural, organizational, functional, biological, and fundamental unit of all known living organisms. This statement sounds simple and evident to any student with some background in the biological sciences. Nonetheless, it took centuries for this concept to be demonstrated, developed, and accepted. Because the cells are too small to be discerned with the naked eye, and because cutting-edge technologies and microscopes did not exist, the very existence of cells was not even surmised until the seventeenth century.

Although there are disputes and controversies regarding the original inventors of telescopes and microscopes, the Italian animal and plant anatomist Marcello Malpighi (1628-1694) was among the first to use a microscope to investigate and describe thin slices of animal tissues and to describe the development of chick embryo. He also suggested that plant tissues contain structural units called “utricles” (later to be called “cells”). He was also among the first to observe red blood cells under a microscope. Because of his work, many microscopic anatomical structures were named after him including the Malpighi layer (skin layer), Malpighian corpuscles and Malpighian pyramids in the kidney, Malpighian bodies or Malpighian corpuscles of the spleen as well as the Malpighian tubules in the excretory system of insects. The botanical family Malpighiaceae was also named after him [1].

**Sami Dridi**

Later on, the Dutch scientist Anton Van Leeuwenhoek (1632-1723) became an expert in making superior lenses and building microscopes with a higher magnifying power of 270x. He was the first to see and describe bacteria, protozoa, rotifers, and hydra. He also described the circulation of blood corpuscles in capillaries as well as mammalian sperm cells.

The work of Leeuwenhoek was confirmed and further developed by the English architect and scientist Robert Hooke (1635-1703), who published Micrographia in 1665. Using his microscope, Hooke examined a thin slice obtained from dried cork and observed the pores where he decided to call them cells [2]. Later, the French physician, botanist, and physiologist René Joachim Henri Dutrochet (1776-1847) was given credit for discovering plant and animal cells and the actual discovery of the process osmosis. He wrote that all animal and plant tissues were "aggregates of globular cells" [3]. Robert Brown, the Scottish botanist (1773-1858), made a critical contribution to cell biology *via* the earliest detailed description of the cell nucleus and cytoplasm [4]. In 1839, the Czech anatomist and physiologist Johann Evangelist Purkinje coined the term "protoplasm" to describe the content of cells. The German botanist Matthias Jacob Schleiden (1804-1881) extended the studies begun by Robert Brown on the structure and function of the cell nucleus (which Schleiden called a "cytoblast") and was the first to describe the nucleoli.

In 1839 and in association with Theodor Schwann (a German physiologist, 1810-1882) and Rudolf Ludwig Carl Virchow (a German physician and biologist), Schleiden established the cell theory, which contains three tenets: 1) all organisms are composed of one or more cells, 2) the Cell is the basic unit of structure and organization in organisms, and 3) cells arise from pre-existing cells. The abovementioned cell doctrine was the foundation of modern biology where unprecedented growth of science and knowledge about the Cell, its structural organization and diversity as well as the function of its components parts, has been made. The modern version of the cell theory includes the following additional tenets: 1) energy flow occurs within cells, 2) heredity information (DNA) is passed from Cell to Cell, and 3) all cells have the same basic chemical composition.

This unprecedented understanding is founded on the contributions of thousands and thousands of scientists from across the globe who partook in breakthrough discoveries leading to solutions that make the world of today a better place.

In this chapter, after a brief description of the body, tissues, system, organism, I will describe the structure and function of the major cell organelles with a focus on eukaryotic cells.

## 2.1. From the Cell to the Body

As the cell theory stated, all organisms are composed of one or more cells. Unicellular organisms, such as amoebas, bacteria, yeast, and plankton, consist of only a single cell. Plants and animals are, however, complex multicellular organisms. The structure of the animal body, is arranged into particular systems with their specific functions. Cells together form tissue, which is grouped to form organ. Each body organ has a specific shape and is composed of various types of tissues that provide complex physiologic activities. Usually, two or more organs together with other tissue that provide particular types of body functions are called body organ systems.

### *2.1.1. Organ Systems*

In animals, there are eleven distinguish organ systems: integumentary, nervous, immune and lymphatic, cardiovascular, respiratory, digestive, urinary, reproductive, muscular, skeletal, and endocrine systems.

#### *2.1.1.1. The Integumentary System*

It is the largest organ system and it is formed of three main parts: epidermis, dermis, and subcutis or hypodermis (Fig. **2.1**). Among the skin related structures, we can include hair, nails, sense receptors, glands, claws, declaws, hooves, horns, and feathers (in birds). An important part of the integumentary system is formed by the sebaceous glands, located in the dermis, which produce sebum (oily matter) that helps hydrate the skin. The second gland, sweat gland, plays a key role in thermoregulation. Its primary function is to cool the organism, to a high degree in horses and a lesser degree in swine, sheep and goat. Birds do not have sweat glands, but they do have additional integuments such as beaks, comb, wattles, and feathers. Thus, the integumentary system serves as a barrier to protect the body internal parts from injury, damage, hydration, or invasion by infectious agents and regulate temperature, produce pigments, vitamin D, store nutrients, and provide sensory perception. In birds, the feathers serve also for flight.

#### *2.1.1.2. The Nervous System*

The nervous system is divided into two basic parts: 1) the central nervous system (CNS) containing the brain and the spinal which is known as the control center where information is evaluated and decisions are made, and 2) the peripheral nervous system (PNS) containing the sensory nerves and sense organs which monitor conditions inside and outside the body and communicate this information with the CNS (Fig. **2.2**).

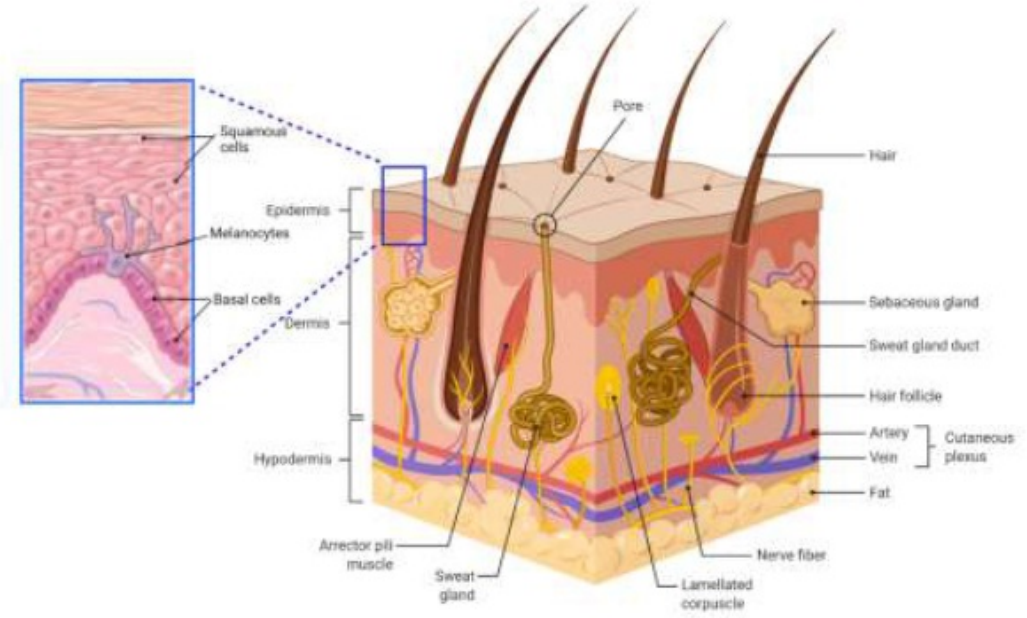


**Fig. (2.1).** Generalized features of integumentary system.

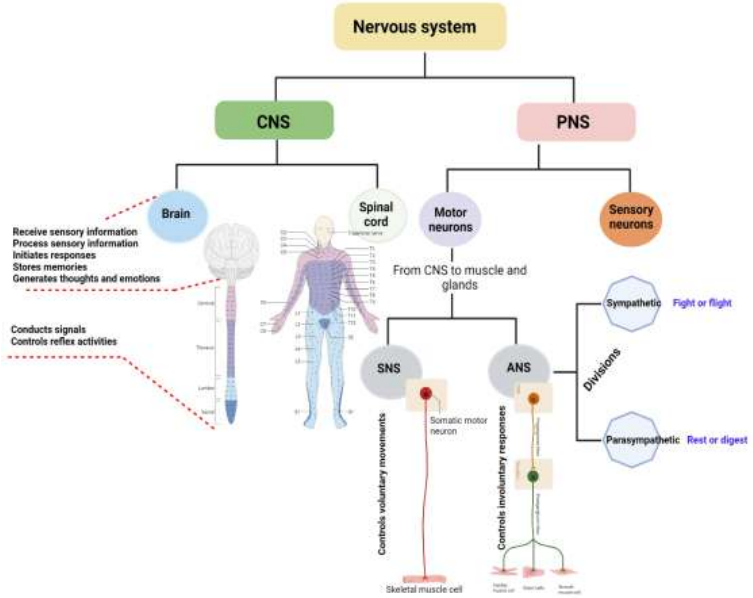


**Fig. (2.2). Generalized structure of the nervous system.** ANS, autonomic nervous system; CNS, central nervous system; PNS, peripheral nervous system; SNS, somatic nervous system.

The PNS contains the somatic nervous system (SNS), which includes all of the voluntary efferent neurons and controls conscious efforts such as skeletal muscle contraction. The autonomic nervous system (ANS), however, includes all the involuntary efferent neurons and controls subconscious efforts such as visceral and cardiac muscles. There are two divisions of the ANS in the body: 1) the sympathetic division, which is responsible for the "fight or flight" responses to stress, stimuli, dangers, emotions, excitement, exercise, and embarrassment. This sympathetic division increases respiration and heart rates and releases adrenaline and other stress hormones such as corticosterone or cortisol and 2) the parasympathetic division, which controls the "rest and digest" responses during relaxation, resting, or feeding. This division functions to decrease respiration and heart rates and increase digestion. The ANS also contains the enteric nervous system (ENS), which controls the digestive organs and regulates digestion. Although it communicates with the CNS *via* the sympathetic and parasympathetic divisions, the ENS works independently without any outside inputs and thereby it is frequently called "brain of the gut", "little brain" or the "second brain" [5 - 7].

The nervous system is thus responsible for collecting, transferring, and processing information as well as consciousness or awareness of living organisms, intelligence and learning. It also detects special senses like vision, taste, smell, hearing, and balance.

#### ***2.1.1.3. The Immune and Lymphatic System***

The immune and lymphatic systems are two closely related organ systems that share several organs and immune-physiological functions. This system plays a key role in defending the animal body against infectious pathogens (virus, bacteria, fungi, *etc*.) and other foreign bodies. It contains several organs, including capillaries, vessels, lymph, lymph nodes, bone marrow, thymus, spleen, and tonsils (lymphoid tissues). Additional to these organs, the lymphatic-immune system in birds contains the Harderian gland and the bursa of Fabricius [8] (Fig. **2.3**). The bone marrow, a soft, spongy, and fatty substance in the cavities of bones, contains blood-forming hematopoietic stem cells, which are a source of erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). The afferent lymph vessels transport the lymph to the lymph node, where it is filtered before it is returned to the circulatory system. The spleen filter and cleans the blood and lymph, recycle the old red blood cells, and store platelets and white blood cells. In the thymus, thymocytes (bone-marrow hematopoietic precursors) mature into T cells which emigrate to form peripheral T cells responsible for directing many parts of the adaptive immune response [9].

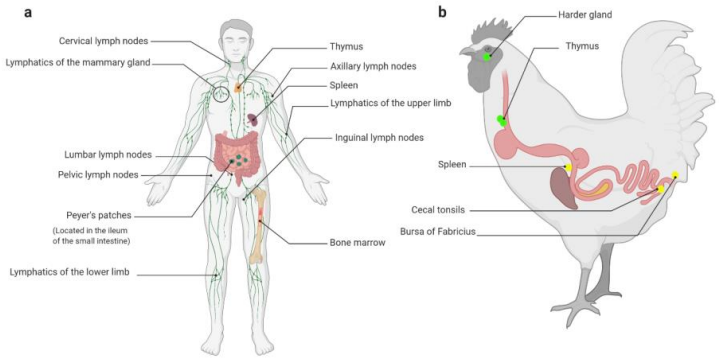


**Fig. (2.3).** Generalized features of the immune-lymphatic system in human **(a)** and birds **(b)**.

## *2.1.1.4. The Cardiovascular System*

The cardiovascular system comprises the heart, blood vessels and the network of the lymphatic system (Fig. **2.4**). Its main function is to transport oxygen, nutrients, hormones, and cellular waste products throughout the whole body. This system is powered by the heart, cone-shaped muscular pumping organ, which uses two primary circulatory loops. The pulmonary circulation transports deoxygenated blood from the right side of the heart (atrium and ventricle) to the lungs, where the blood picks up oxygen and returns to the left side of the heart. The systemic circulation carries highly oxygenated blood from the left atrium and ventricle to all body tissues. This systemic circulation removes waste from body tissues and returns deoxygenated blood to the right side of the heart.

The blood flows away from the heart *via* the arteries (blood vessels with a thick wall around the lumen) and exchanges oxygen and nutrients with the cells of the tissues *via* capillaries (blood vessels with a thin wall around the lumen). The veins carry back the blood to the heart.

The cardiovascular system plays a pivotal role in transportation (oxygen, nutrients, cellular wastes), protection against pathogens *via* white blood cells and transport of antibodies, and regulation and homeostatic maintenance of several internal conditions, including the core body temperature and the body's pH.

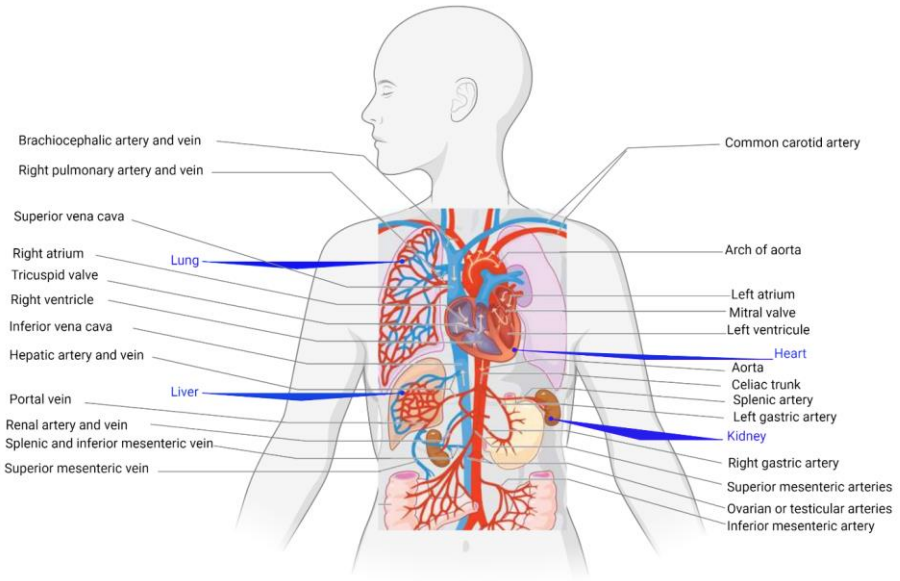


**Fig. (2.4). Generalized features of the cardiovascular or circulatory system.** The systemic circulation (in red) caries oxygenated blood and the pulmonary circulation (in blue) transports deoxygenated blood.

### *2.1.1.5. The Respiratory System*

The respiratory system contains three major parts, including the airway (nose, mouth, pharynx, larynx, trachea, bronchi, and bronchioles), the lung, and the respiration muscles (diaphragm and the intercostal muscles) (Fig. **2.5**). The respiratory system exchanges gases between internal body tissues and the external environment. Oxygen in the air is inhaled from the external environment through the airway into the lung, which helps to pass the oxygen to the blood and tissues. Through vessels, the carbon dioxide is carried back from tissues into the lung and is expelled out of the body.

In comparison with mammals and in addition to the lung, birds also have air sacs (Fig. **2.5**). Depending upon the species, birds have seven or nine air sacs (2 cervical, 2 cranial thoracic, 2 caudal thoracic, 2 abdominal, and 1 interclavicular air sacs). Additionally, birds do not have a diaphragm; instead air is moved in and out of the respiratory system *via* pressure changes in the air sac. Bird lungs do not expand or contract like that of mammals. Mammalian lungs contain alveoli (microscopic sacs) where the exchange of oxygen and carbon dioxide occurs; however bird lungs contain air capillaries (walls of microscopic tubules). Respiration in birds requires two respiratory cycles (inspiration, expiration,

inspiration, and expiration) to move air through the entire respiratory system. In mammals, however, only one respiratory cycle is required.

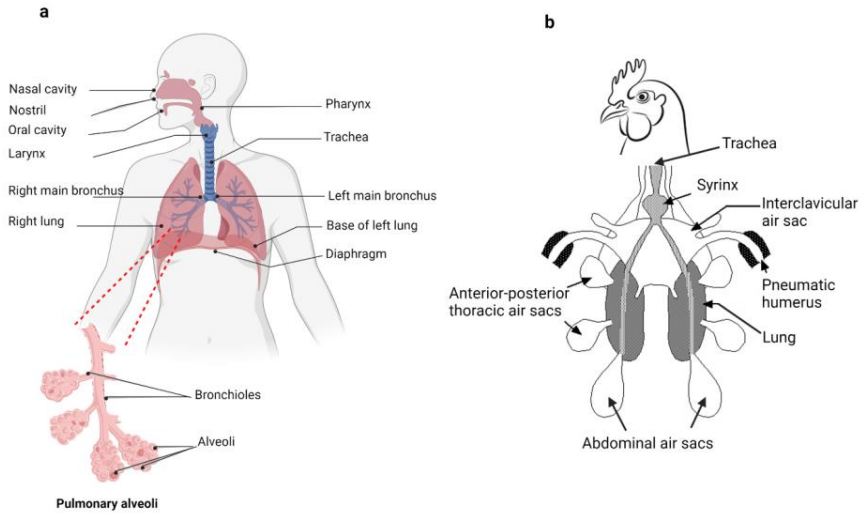


**Fig. (2.5).** Diagram of the respiratory system in human **(a)** and birds **(b)**.

## *2.1.1.6. The Muscular System*

The muscular system is responsible for the movement of the animal body. In humans, for example, there are 700 named muscles attached to the bones of the skeletal system. Each of these muscles is a discrete organ constructed of skeletal muscle tissue, blood vessels, tendons, and nerves. There are three types of muscles, including visceral, cardiac, and skeletal muscle (Fig. **2.6**).

The visceral muscle also known as smooth muscle is located inside of the gastrointestinal tract (stomach and intestines) as well as in the blood vessels. Its function is to move substances through the organ. It is an involuntary muscle because it is controlled by the unconscious part of the brain.

The cardiac muscle is located only in the heart and is responsible for pumping blood throughout the body. It is an involuntary muscle and is intrinsically controlled.

The only voluntary muscle is the skeletal muscle, which is controlled consciously. Its primary function is to contract and move the other parts of the body for every physical action (speaking, walking, or writing). The secondary function is to

maintain the posture and body position. The final function of the muscular system is to generate body heat *via* shivering thermogenesis.

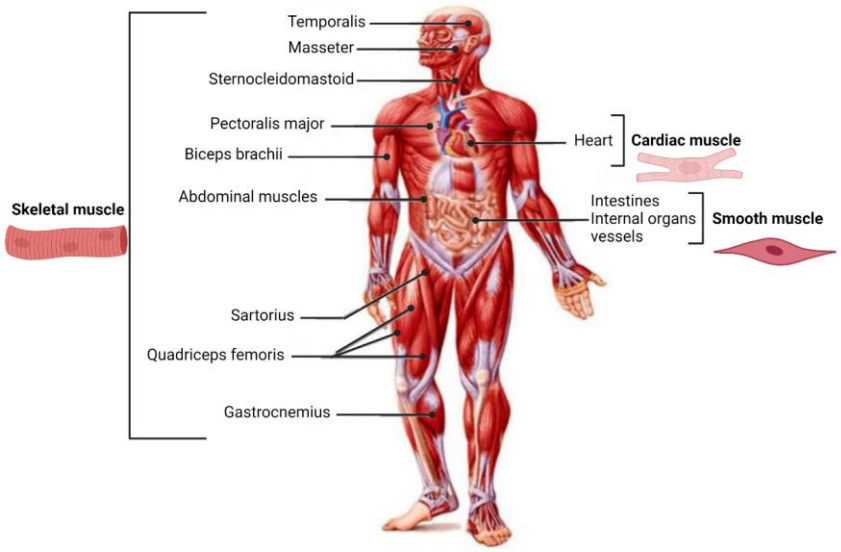


**Fig. (2.6). The human muscular system.** Adapted from McGraw-Hill Education.

## *2.1.1.7. The Skeletal System*

The skeletal system encompasses all of the bones and joints in the body. For an adult human, this system contains 206 individual bones arranged into axial and appendicular skeleton (Fig. **2.7**). The axial skeleton, which runs along the body's midline axis, is made up of 80 bones in the skull, hyoid, auditory ossicles, sternum, vertebral column, and ribs. The appendicular skeleton contains 126 bones in the upper limbs, lower limbs, pelvic girdle, and pectoral girdle.

The bones can be categorized into four types (Fig. **2.7**): 1) long bones, such as femur, tibia, fibula, and metatarsals, are responsible for the bulk of our height as adults. They contain a medullary cavity in the center and serve as storage for bone marrow. 2) Short bones, like the tarsal bones of the foot, are often cubed or round in shape. 3) Flat bones, such as the occipital bones of the cranium and the hip bones, do not have a medullary cavity. 4) Irregular bone contains the sacrum, vertebrae and coccyx of the spine as well as the zygomatic bones of the skull. 5) The sesamoid bones, the patella and the pisiform bone, protect the tendon from stress.

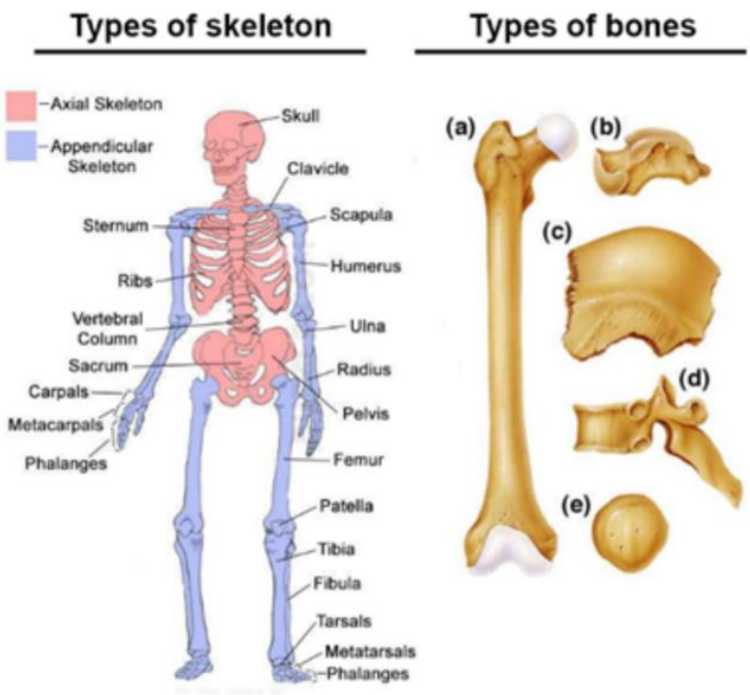


**Fig. (2.7). Diagram of the skeletal system. a)** Long bone, **b)** short bone, **c)** flat bone, **d)** irregular bone, and **e)** sesamoid bone. Adapted from www.pinterest.com

The function of the skeletal muscle is to form a solid framework that supports and protects the body's organs, such as the skull and the brain. It plays a key role in hematopoiesis (production of red and white blood cells from the bone marrow). It is also involved in the storage of many different types of essential substances such as calcium to facilitate the growth and repair of the body.

### *2.1.1.8. The Urinary System*

The urinary system consists of the kidney and the urinary tract, which contains the ureter, urinary bladder, and urethra (Fig. **2.8**). The kidney filters the blood to remove wastes and produce urine. The urinary tract acts as a plumbing system to drain, store, and release urine during urination. This system plays a key role in maintaining the homeostasis of water, ions, pH, osmolarity, and blood pressure (see chapter 4). In comparison to mammals, birds do not have a bladder, but they

do have a cloaca where solid and liquid wastes are mixed together. The major end product of nitrogen catabolism in birds is uric acid, accounting for 70-80% of the nitrogen excreted [10].

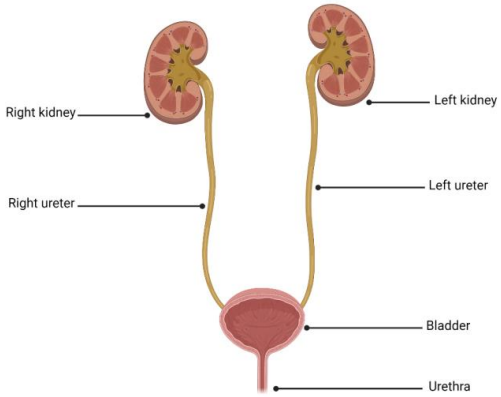


**Fig. (2.8).** Diagram of the urinary system.

### *2.1.1.9. The Digestive System*

The digestive system contains a group of organs working together to convert food into energy and basic nutrients to feed the entire body. The main alimentary canal, where food passes through, comprises the oral cavity, pharynx, esophagus, stomach, small and large intestines. Several other accessory organs such as the teeth, tongue, salivary glands, liver, gallbladder, and pancreas help the body digest food (Fig. **2.9**).

This system has six major functions, including ingestion, secretion, mixing and movement, digestion, absorption, and excretion (see chapters 7, 8, and 9, sections 7.3, 8.2, and 9.2.).

The anatomy and function of the digestive system differ between species. For instance, in a*via*n species, known as modified monogastric, the beak is the prehension tool. Additionally, although humans and birds both have small and large intestines as well as the esophagus, birds have proventriculas compared to stomachs in humans. They also have gizzards compared to teeth in humans. The proventriculus is a glandular stomach however the gizzard is a muscular stomach for mechanical breakdown. The digestive system of birds comprises also a crop

for feed storage and moistening and a cloaca. Unlike monogastrics, the digestive system achieved its highest complexity in anatomy and function in ruminants such as cows, sheep, and goats. The term ruminant was derived from the Latin word "*ruminare*" which means "chew again". Thus ruminants chew their cud by regurgitation of ingested materials. Their stomach contains four compartments, including the rumen, reticulum, omasum, and abomasum (Fig. **2.9**). As it contains many anaerobic bacteria, the rumen, also known as anaerobic fermentation vat, secretes enzymes that digest the consumed food and produce volatile fatty acids and ammonia. The rumen also synthesizes amino acids and water-soluble vitamins. The reticulum functions to eliminate foreign materials. The primary function of the omasum is to further grind and break down feed. Fluids and small particles flow through the omasum to the abomasum, true gastric stomach, where acid digestion rather than microbial digestion takes place. The acidity in the abomasum kills the rumen microbes in the digesta before they move to the small intestine.

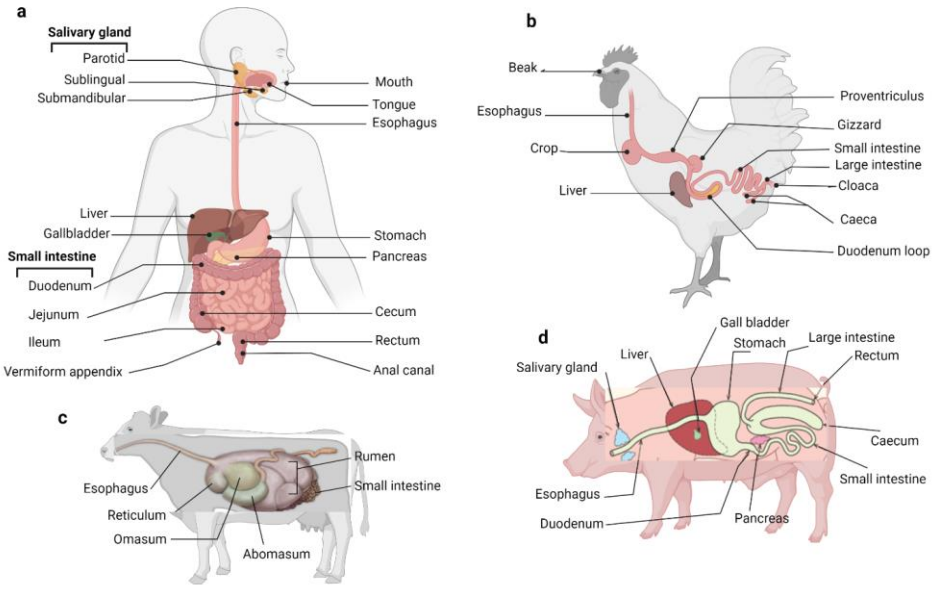


**Fig. (2.9). The general features of simple monogastric, modified gastric and complex ruminant digestive tract. a)** Human, **b)** a*vian*s, **c)** bovine and **d)** porcine.

## *2.1.1.10. The Reproductive System*

The reproductive system is different in males and females (Fig. **2.10**). The female reproductive system includes the ovaries, fallopian tubes, uterus, vagina, vulva,

mammary gland, and breasts. Its main function is to facilitate the fertilization of ova by sperm and supports the development of offspring during pregnancy (gestation in animal) and infancy. The male reproductive system, however, contains the scrotum, testes, spermatic ducts, seminal vesicle, prostate gland, and penis. Its primary function is to produce sperm, the male gamete, and the other components of semen.

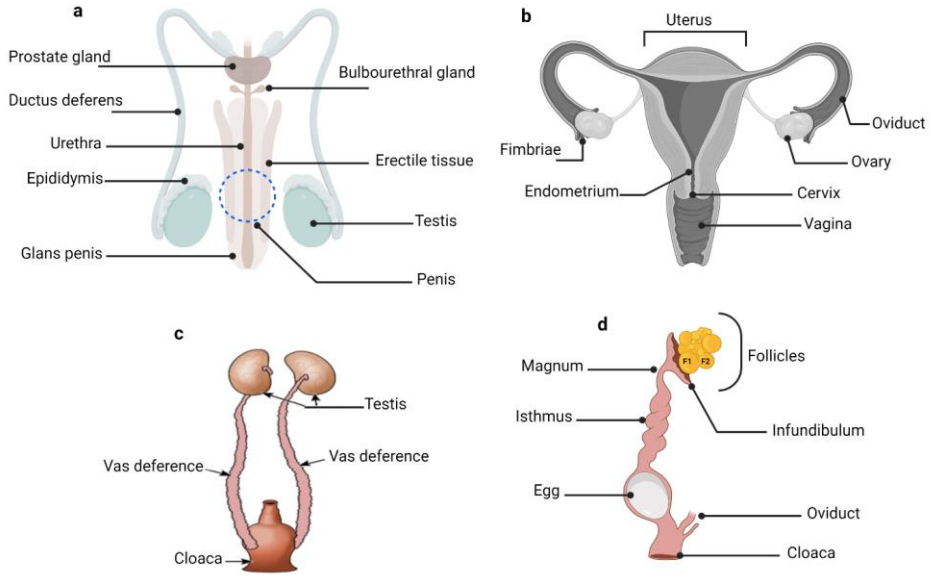


**Fig. (2.10). The reproductive system. a)** Man, **b)** woman, **c)** roster, and **d)** hen.

Similarly, the reproductive system differs between mammals and a*via*n species. Hens lay eggs instead of live birth in mammals, they do not have mammary glands, and only their left oviduct and ovary are functional. Male birds do not have a penis, and their tests are internal *versus* externals in mammals. In birds, females are heterogametic sex (ZW) and males are homogametic (ZZ). Female birds determine the sex of offspring [11, 12].

## ***2.1.1.11. The Endocrine System***

The endocrine system comprises all the glands and their related hormones. The glands are controlled directly by stimulation from the CNS and from the peripheral organ-producing hormones. In response to various stimuli (nutritional status, environment, stress, *etc*.), glands (pituitary, pineal, thyroid, parathyroid,

adrenal, pancreas, gonads, thymus, *etc*.) (Fig. **2.11**) and several other organs secrete hormones in the bloodstream, which affect any cell with a receptor for a particular hormone (Table **2.1**). By affecting cells in several organs or throughout the entire body, the main function of the endocrine system is to maintain the body's homeostasis as well as the function of other systems, including reproduction, heart rate, digestion, *etc*.

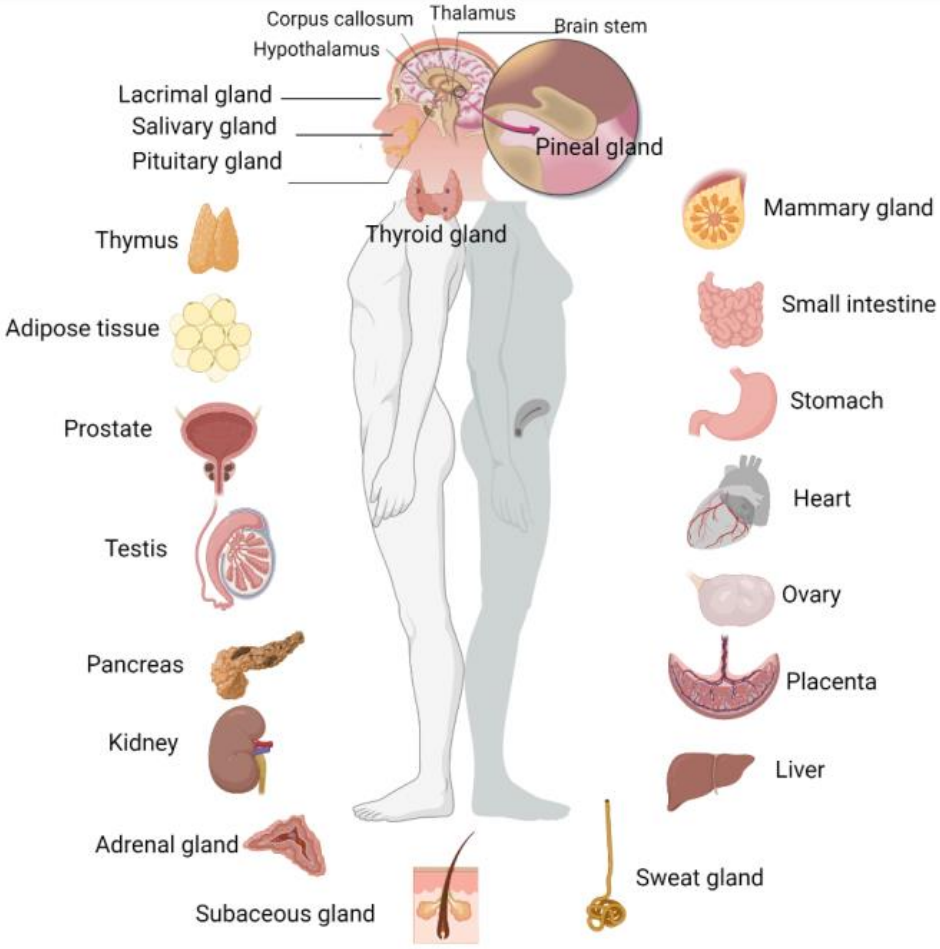


**Fig. (2.11).** The endocrine system.

**Table 2.1. List of some important polymers and their roles.**

| Organs/Glands | Hormones and/or Peptides | Function |
|---|---|---|
| Hypothalamus | Orexin [13] | Appetite and wakefulness |
| | Antidiuretic hormone (ADH) [14] | Thirst regulation |
| | Oxytocin [15] | Feeling and emotion |
| Pituitary gland | Thyroid-stimulating hormone [16] | Thyroid hormone regulation |
| | Prolactin [17] | Mammary gland growth |
| | Follicle-stimulating hormone [18] | Oocyte and ovarian follicle control |
| | Luteinizing hormone [19] | Induces ovulation |
| | Adrenocorticotrophic hormone [20] | Control corticosteroid production |
| | Growth hormone [21] | Induce growth and regulate metabolism |
| Pineal gland | Melatonin [22] | Regulates circadian rhythm (biological clock) |
| Thyroid gland | Thyroid hormone (T3 and T4) [23] | Regulate energy metabolism and heat production |
| Parathyroid gland | Parathyroid hormone [24] | Regulate calcium in bone tissues |
| Thymus | Thymosin, thymulin [25] | Maturation of T-lymphocytes |
| Adrenal cortex | Mineralcorticoids [26] | Control of glucose and glycogen metabolism |
| Pancreas | Insulin and glucagon [27] | Regulates glucose levels |
| Testes | Testosterone [28] | Regulates male reproductive system |
| Ovaries | Estrogen and progesterone [29] | Regulate female reproductive system |
| Kidney | Erythropoietin [30] | Controls the production of red blood cells |
| Liver | Angiotensinogen [31] | Regulates blood pressure |
| Stomach | Gastrin [32] | Regulates digestion |
| Small intestine | Secretin, cholecystokinin [33] | Regulates digestion and feed intake |
| Adipose tissue | Leptin, visfatin [34, 35] | Regulate energy homeostasis |
| Placenta | Leptin [36] | Regulates energy homeostasis |

Hormones are classified into two classes: 1) water-soluble hormones such as insulin, epinephrine, and oxytocin. These hormones are unable to pass through the phospholipid bilayer of the cell plasma membrane and are therefore dependent upon their receptors on the cell surface. 2) Lipid-soluble hormones include steroid hormones such as testosterone, estrogens and glucocorticoids. As they are lipid-soluble, these hormones pass through the phospholipid bilayer of the cell plasma membrane and bind directly to its receptors inside the cell nucleus.

### *2.1.2. Tissues*

As mentioned above, every organ in the body is made up of two or more tissues,

aggregates of similar cells (shape and function) and cell products forming a definite kind of structural material with a specific function in a multicellular organism. In humans and other animals, there are four basic types of tissues: epithelial, connective, muscular, and nervous tissue.

### *2.1.2.1. Epithelial Tissue*

Epithelial tissue consists of tightly packed sheets of cells (epithelial cells) that cover the body surface and form the lining for most internal cavities. Depending on where they are in the body, they have different shapes (Fig. **2.12**): 1) Flat shape for squamous epithelial cells which are usually found lining surface that require a smooth flow of fluid such as blood vessels, or lining areas that require a thin surface for molecules to pass through like the air sacs of the lung. 2) Cuboid shape for cuboidal epithelial cells, which are typically found in organs that secrete or absorb substances such as the kidney and glands. 3) Columnar epithelial cells are long and thin. They are typically found in organs that secrete mucus, such as the stomach and intestine. 4) Ciliated columnar epithelial cells are found in the upper respiratory tract, the fallopian tubes, the uterus, and the central part of the spinal cord. They are covered by cilia in their apical surface. Additionally, epithelial cells can be described as being either simple with only one layer or stratified with many layers stacked on top of each other such as the skin.

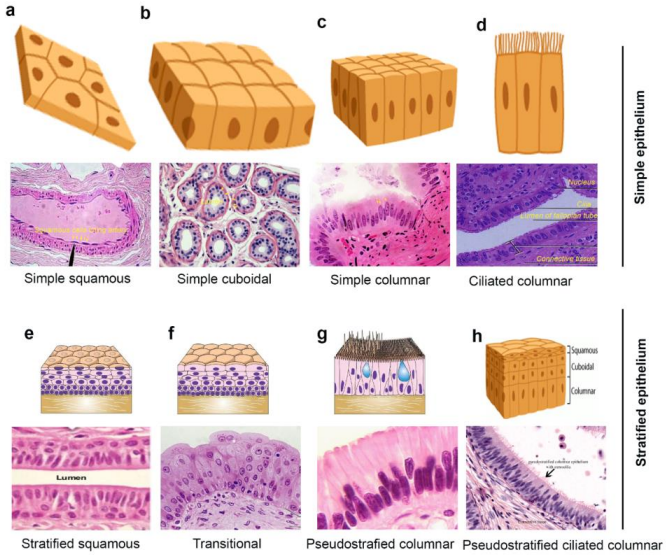


**Fig. (2.12). Different shapes of simple and stratified epithelium. a)** Simple squamous lining the artery; **b)** simple cuboidal, pancreas; **c)** simple columnar, digestive tract; **d)** ciliated columnar, fallopian tube; **e)** stratified squamous, salivary gland; **f)** transitional, bladder; **g)** pseudostratified columnar, male genital tract; and **h)** pseudostratified ciliated columnar, epididymis.

Epithelial cells are polarized with an apical (top) side that faces the inside of a cavity or the outside of a structure and is usually exposed to fluid or air. The bottom side or basal side faces the underlying cells. The epithelial cells are joined together by tight junctions that hold them tightly together to prevent leaks. The epithelial cells exchange nutrients *via* specialized gap junctions. The major function of epithelial cells includes protection, secretion, absorption, and filtration.

### *2.1.2.2. Connective Tissue*

As the name implies, connective tissue serves a connecting function. It consists of cells suspended in an extracellular matrix, which is made up of protein fibers such as collagen and fibrin in a solid, liquid, or jelly-like ground substance. Connective tissue supports and binds other tissues in the body.

Connective tissue fibers and matrix are synthesized by specialized cells called fibroblasts and are divided into three types, including collagenous (to strengthen the connective tissue), elastic (to help stretch the connective tissue), and reticular fibers (to join connective and other tissues). There are three categories of connective tissues (Fig. **2.13**): 1) Loose connective tissue, the most common tissue in vertebrates, holds organs in place and attaches epithelial tissue to other underlying tissues.

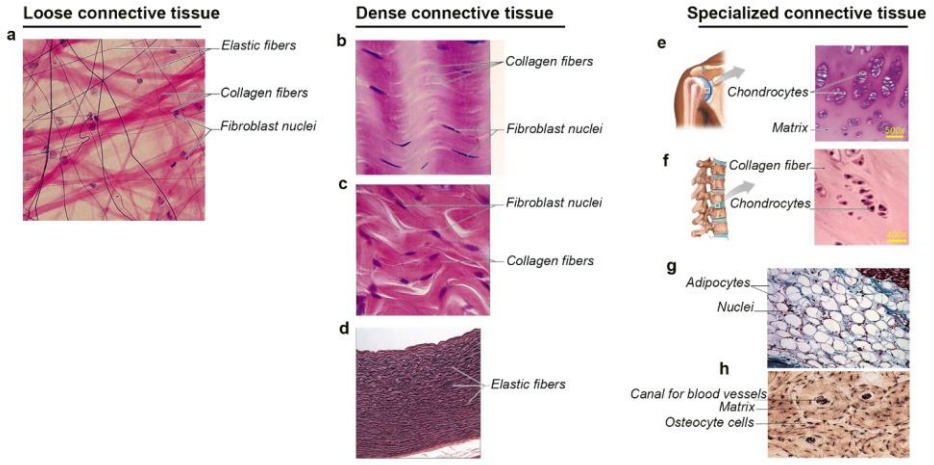


**Fig. (2.13). Different types of connective tissues.** a) Areolar connective tissue (loose connective tissue), b) dense regular connective tissue from a tendon, c) dense irregular connective tissue from the dermis of the skin, d) elastic connective tissue in the wall of the aorta, e) Hyaline cartilage connective tissue from the shoulder joint, f) fibrous cartilage from the intervertebral disc, g) adipose tissue, and h) bone connective tissue.

2) Dense or fibrous connective tissue is found in tendons and ligaments, which connect muscles to bones and bones to each other, respectively. It also forms a protective capsule layer around organs such as the liver and kidney. Dense connective tissue can be categorized into dense regular (tendons and ligaments), dense irregular (dermis of the skin), and elastic connective tissue (artery, trachea, and vocal cord). 3) Specialized connective tissues include a number of different tissues with specialized cells and unique ground substances. Some tissues are solid and strong, while others are fluid and flexible. For instance, adipose tissue, cartilage, bone, blood, and lymph are all specialized connective tissues.

### *2.1.2.3. Muscle Tissue*

Because of their "excitable" cells, muscle tissue is capable of contraction. Similar to connective tissue, muscle tissue is also the most abundant tissue in most animals. It contains microfilaments that are composed of contractile proteins, namely actin and myosin which are responsible for muscle contraction and movement. There are three types of muscle tissue (Fig. **2.14**):

#### *2.1.2.3.1. Skeletal Muscle*

Skeletal muscle is a striated (striped) muscle that is attached to bones by tendons, controlled by the peripheral nervous system (PNS), and is associated with body's conscious and voluntary movements. Skeletal muscle cells are covered and protected by connective tissues. Blood vessels

and nerves run through the connective tissue and supply the muscle cells with oxygen, nutrients, and nerve impulses that induce contractions. Skeletal muscle can be organized from top to bottom in four major groups, including head and neck muscles (facial expression, chewing, neck movement), trunk muscle (chest and abdominal muscles), upper extremity muscle (deltoid and biceps responsible for the movement of shoulders, arms, hands, and fingers), and lower extremity muscle (quadriceps, hamstring, groin, and calf muscles responsible for the movement of legs, ankles, feet, and toes) (Fig. **2.14a-c**).

#### *2.1.2.3.2. Visceral (Smooth) Muscle*

Visceral muscles are mainly found in the walls of blood vessels and the digestive tract as well as in many other hollow organs (Fig. **2.14d, e**). Visceral muscles are also called smooth muscles because of lack of cross striations. They are involuntary and regulated by the autonomic nervous system (ANS). Visceral muscles contract slower than skeletal muscle however, the contraction could be sustained for a longer period. They are two types of smooth muscle, including smooth rhythmic muscles which contract periodically and spend most of the time

in a relaxed state, and tonic smooth muscles which remain contracted for the majority of the time and only relax periodically. Organs of the cardiovascular, respiratory, digestive, and reproductive systems are lined with smooth muscle.

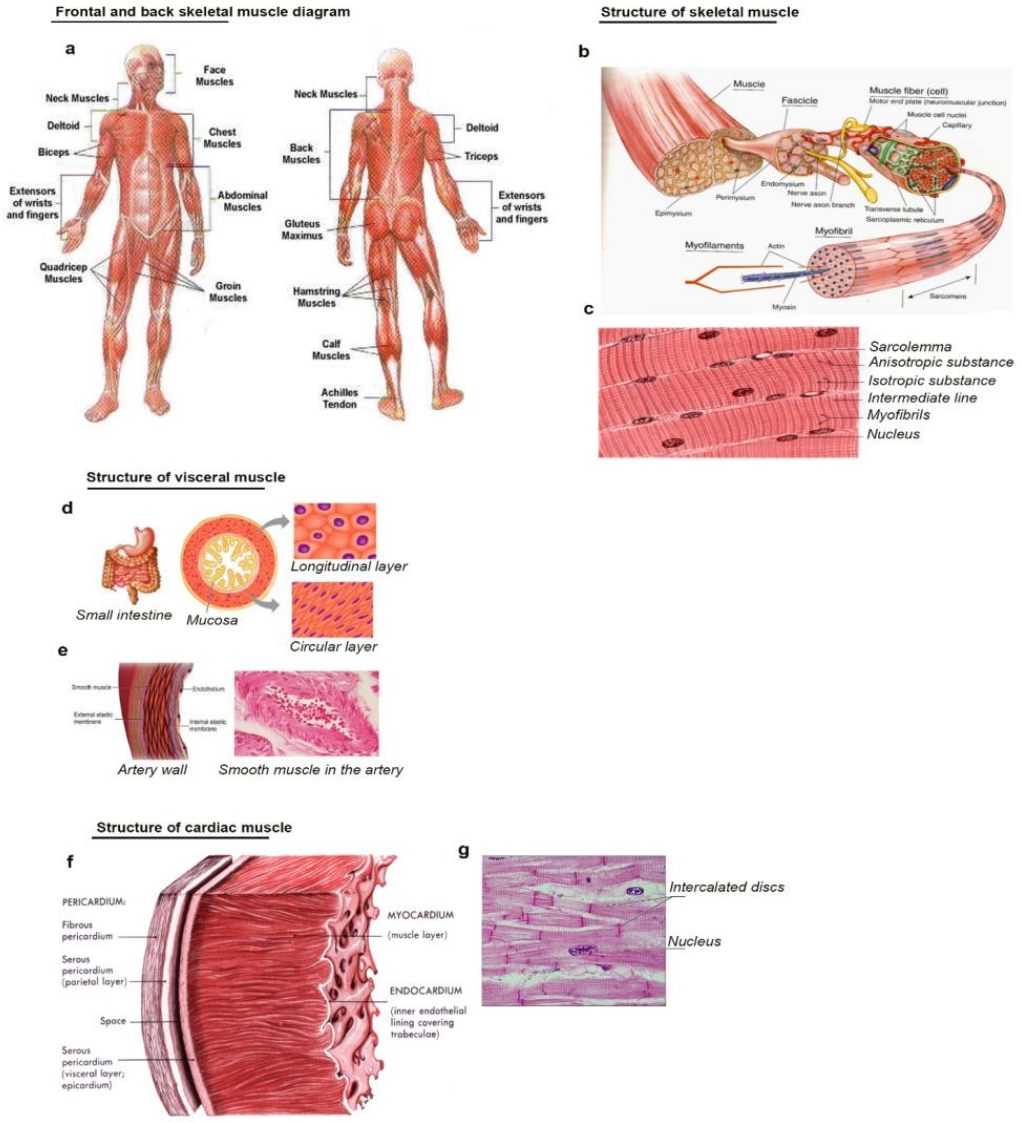


**Fig. (2.14). Different types of muscle tissue. a)** Frontal and back muscle diagram, (**b** and **c**) skeletal or striated muscle tissue structure showing that skeletal muscle has striated, tubular, and multinucleated fibers, (**d** and **e**) structure of smooth muscle from the small intestine and the wall of the artery, respectively. It shows that smooth muscle has spindle-shaped, non-striated and uninucleated fibers. (**f** and **g**) structure of cardiac muscle which has striated, branched, and uninucleated fibers.

*2.1.2.3.3. Cardiac Muscle*

Cardiac muscles are found only in the walls of the heart. They are branched and striated. Cells are joined to one another by intercalated discs, which allow the synchronization of the heart contraction and beat (Fig. **2.14f and g**). The heart wall consists of three layers: epicardium, myocardium, and endocardium.

### *2.1.2.4. Nervous Tissue*

Nervous tissue is the main component of the nervous system, which includes the brain, spinal cord, and nerves. It is involved in integrating and sensing stimuli- external or internal cues- and processing, transmitting, and communicating information. Nervous tissue contains two categories of cells, including nerve cells (neurons) and neuroglia known as glia (Fig. **2.15**).

The neurons are highly specialized nerve cells that convey information very rapidly across long distances through generating conducted nerve impulses or action potentials. There are several types of neurons, including motor neurons, sensory neurons, and relay neurons. A typical neuron consists of an enlarged part called the cell body (or perikaryon), which contains the nucleus and dendrites that receive the nerve impulse and an axon. Depending on the processes, neurons can be unipolar (having an exon going towards and from the soma), which is mainly found in insects, bipolar (having two processes, an axon and dendrite, and are usually seen in sensory function such as the bipolar cells of the retina), or multipolar which contain one axon and multiple dendrites and commonly seen in motor and sensory functions

The neuroglia acts mainly to support neuronal function. There are several types of glia cells: 1) Astrocytes (also known as astroglial cells), which supply neurons with nutrients, help migration of neurons during brain development, aid formation of the blood-brain barrier (BBB), maintain appropriate balance of $Ca^{2+}$ and $K^{+}$ ions, and remove excess transmitters.

1. Ependymal cells form the lining of the ventricles of the brain and central canal of the spinal cord and aid formation and circulation of cerebrospinal fluid (CSF).
2. Microglial cells play a key scavenging role by clearing debris and dead cells and thereby protecting the central neurons from diseases.
3. Oligodentrocyte cells are found in the CNS and produce myelin sheath around several adjacent axons of the CNS neurons.
4. Schwann cells are found in the PNS and form myelin around a portion of a single axon only and they help with the regeneration of PNS axons.

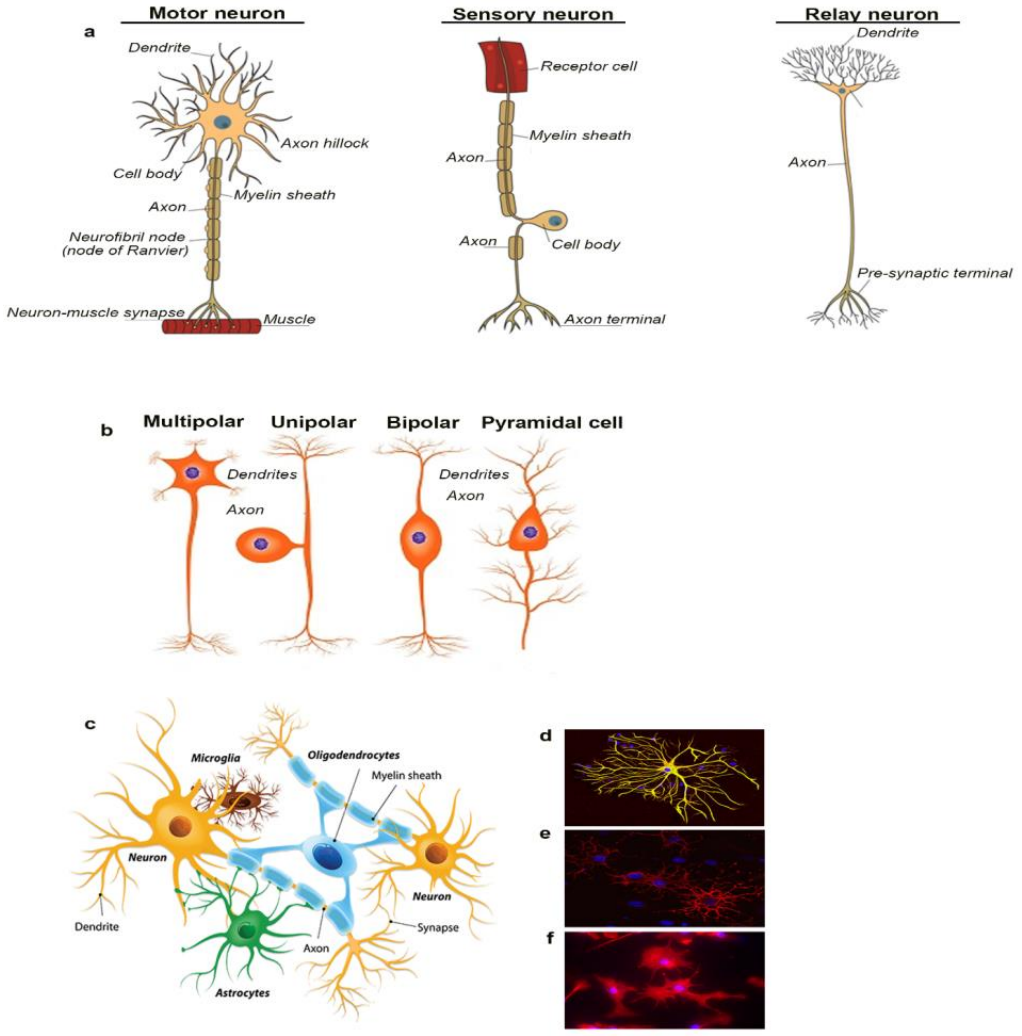


**Fig. (2.15). General features of nerve tissue**. (**a** and **b**) Different types of neurons, (**c**) structure of astrocytes and oligodendrocytes, (**d**) immunofluorescence staining of astrocyte cell from rat brain grown in tissue culture and double-stained with antibodies against the glial fibrillary acidic protein (GFAP, red) and vimentin (green) which make the astrocyte cell appears yellow. The nucleus of the Cell appears blue with DAPI staining. (**e**) Immunofluorescence staining of rat oligodendrocytes using oligodentrocyte marker O1 antibody (red) and the nuclei were stained with DAPI. (**f**) Immunofluorescence picture of primary microglial cells isolated from mouse brain

### *2.1.3. Cells*

Fundamentally, there are three types of cells: prokaryotic, archaeans, and eukaryotic cells. Cells of all multicellular organisms are eukaryotic and their name comes from the Greek *eu*, meaning "well or true" and *karyo*n indicating "kernel or nucleus". In contrast, prokaryotic (*i.e.* before nucleus) cells such as bacteria lack a membrane-enclosed nucleus and thereby do not have a defined nucleus. Archaea were classified in the past as bacteria and were called archaebacterial, but it was discovered that they have a distinct evolutionary history and biochemistry (more complex RNA polymerase, no peptidoglycan in the cell wall, and different membrane lipid bonding from bacteria). Because this ebook addresses comparative biochemical nutrition in animals, all discussions of cellular structure and function in this and subsequent chapters pertain to eukaryotic cells.

Although animal cells vary considerably in size (from 1 to 100 μm), shape, organelle composition, and physiological role, they have a common structure and similar organization (Fig. **2.16**). All animal cells have a plasma membrane and nucleus, and most of them contain an endoplasmic reticulum, Golgi apparatus, mitochondrion, and several other organelles, including centrioles, lysosomes and peroxisomes. In addition, they comprise ribosomes macromolecules made of both RNA and proteins. Whether ribosomes are considered as organelle or not, it is a debate matter. Originally, the term organelle referred to only membranous structures however it has come to mean any well-defined subcellular structure that performs a particular function (Table **2.2**). Yet it is noteworthy that animal cells do not have a cell wall or chloroplast, but they do have small vacuoles to store nutrients or water.

#### ***2.1.3.1. The Plasma Membrane***

The plasma membrane also called the cell membrane or plasmalemma is composed of a phospholipid bilayer embedded with protein molecules (Fig. **2.17**). It forms a boundary that separates the living contents of the Cell (cytoplasm and organelles) from the surrounding external environment. It regulates the passage of molecules into and out of the cytoplasm, and in some tissues such as the nervous tissue, it is involved in intracellular communication. In the apical surface of secretory and absorptive tissues, portions of the plasma membrane are modified to form microvilli (fingerlike projections) which greatly increase the surface area of the Cell and provide for the increased exchange of materials across the plasma membrane.

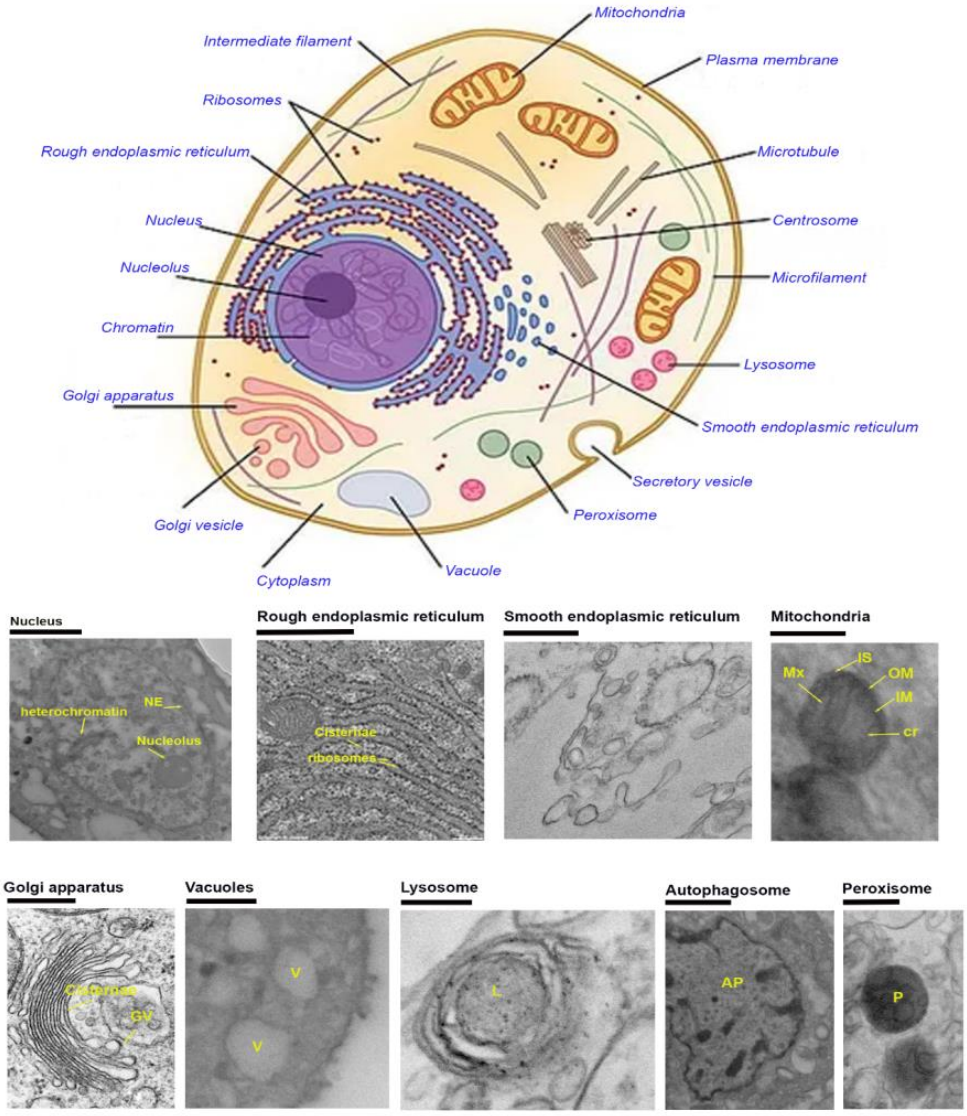


**Fig. (2.16). Structure and composition of eukaryotic Cell.** The upper panel is a cartoon image of animal cell structure and composition. The lower panel is transmission electron micrograph showing different organelles of porcine intestinal enterocytes (IPEG-J2) cells. AP, autophagosome; Cr, cristae; GV, Golgi vacuole; IM, inner membrane; IS, intermediate space; L, lysosome; Mx, matrix; NE, nuclear envelop; OM, outer membrane; P, peroxisome; V, vacuole. Sections were viewed at 100 kV, with a transmission electron microscope (JEM-1011, JEOL, Tokyo, Japan) at the Institute for Nanoscience and Engineering at the University of Arkansas, Fayetteville.

**Table 2.2. Structure and composition of eukaryotic cell.**

| Organelle | Composition | Function |
|---|---|---|
| Plasma membrane | Phospholipid bilayer with embedded proteins | Defines cell boundary and regulates molecule passage into and out of the cell |
| Nucleus | Nuclear envelope, nucleoplasm, nucleoli, and chromatin | Storage of genetic information and synthesis of RNA/DNA |
| Endoplasmic reticulum | Membranous flattened channels and tubular canals | Synthesis and/or modification of proteins and other substances, and distribution by vesicle formation |
| Golgi apparatus | Stack of small membranous sacs | Processing, packaging, and distribution of proteins and lipids |
| Mitochondrion | Inner membrane bounded by an outer membrane | Cellular respiration |
| Cytoskeleton | Microtubules, intermediate filaments, and actin filaments | Shape of cell and movement of its parts |
| Centriole | A self-replicating, minute, fibrous, cylindrical-shaped organelle near the nucleus. It contains 9 pairs of microtubules | Nuclear division |
| Lysosomes | Membranous vesicle containing digestive enzymes | Intracellular digestion |
| Autophagosomes | Double-membraned vesicles that contain cellular material including organelle slated to be degraded | Intracellular digestion |
| Peroxisomes | Membranous vesicle containing specific enzymes such as catalase and peroxidase | Essential metabolic functions, decomposition of fatty acids and hydrogen peroxide |
| Ribosomes | RNA and associated proteins | Protein and polypeptide synthesis |
| Flagelle and cilia | Extension of the cell membrane containing microtubules | Cell movement |

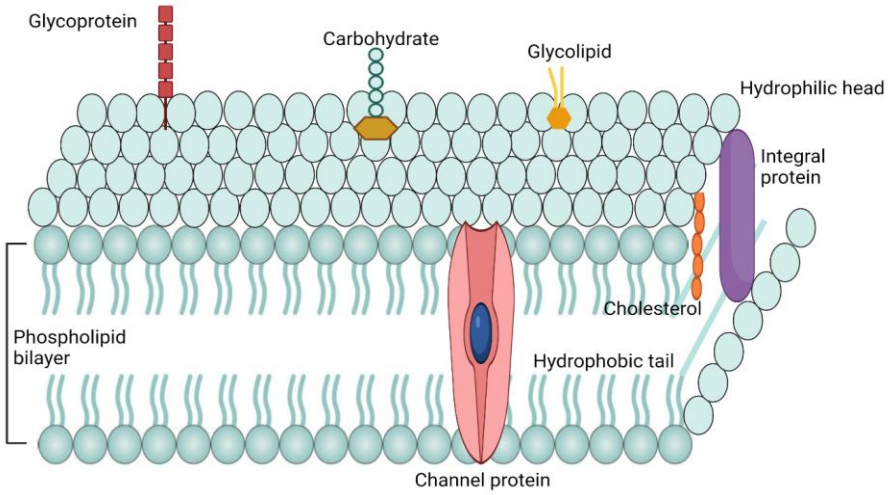


**Fig. (2.17). Structure and composition of the cell membrane.** Proteins of the cell membrane is divided into two groups: Integral proteins (directly incorporated within the lipid bi-layer) and Peripheral proteins (loosely associated with membrane surface).

The plasma membrane of neighboring cells in a tissue frequently exhibits specialized junctional regions that play a role in cell-cell adhesion/communication and in intercellular transport. The most common of these junctions are tight junction (zonula occludens), intermediate junctions or belt desmosomes (also known as zonula adherens), spot desmosomes (macula adherens), gap junctions (connexons or nexuses), and plasmodesmata.

### ***2.1.3.2. The Nucleus***

The nucleus is the largest cellular organelle with a diameter of about 5 μm. The nucleus is separated from the cytoplasm by the nuclear envelope which is composed of two membranes (an inner and an outer membrane) that appear to be a dynamic structure. These membranes are continuous channels with the endoplasmic reticulum, which make possible communication between the nucleus and the cytoplasmic matrix. At various positions, the outer and inner membranes fuse to form the nuclear pores of sufficient size (~ 100 nm), allowing the bidirectional transport of proteins and ribosomal subunits.

The matrix held within the nuclear envelope is composed of chromatin plus all the enzyme and minerals necessary for the activity of the nucleus. Condensed regions of the chromatin are called nucleoli, in which are found not only DNA and its associated alkaline proteins (histones) but also a considerable amount of RNA. Thousands of protein-coding genes are encoded within the nuclear DNA of the

Cell. The protein synthesis occurs in phases, referred to as transcription, translation, and elongation, each of which requires DNA and/or RNA activity, and that will be reviewed in chapter 7 (section 7.4).

### ***2.1.3.3. Mitochondria***

Mitochondria is the energy-related membranous organelle that specializes in converting energy to a form the Cell can use, and they are often called the powerhouse of the Cell. Mitochondria are usually 0.5 to 1 μm in diameter and 2 to 5 μm in length however, the size and shape of the mitochondria in different tissues vary according to the function(s) of the tissue. For instance, mitochondria are held tightly among the fibers in the muscle, but they appear spherical-shaped with freely moving in the liver. Each mitochondrion in the cytoplasm is bordered by two membranes: the outer membrane, which is smooth and porous, and the inner membrane, which contains numerous cristae (invaginations). The space between neighboring cristae is known as the mitochondrial matrix. The inner membrane contains enzymatic complexes essential for the electron transport (respiratory) chain by which most cellular adenosine triphosphate (ATP) is produced. The mitochondria matrix contains various metabolic enzyme systems involved in catalyzing reactions of the Krebs cycle, fatty acid oxidation, oxidative decarboxylation and carboxylation of pyruvate, and certain reactions of amino acid metabolism, which will be discussed in depth in chapters 6 and 7.

Although the nucleus contains most of the Cell's DNA, the mitochondrial matrix encompasses 13 protein-encoding genes and ribosomes that are vital for oxidative metabolism [37]. Interestingly and unlike the nuclear genes, these mitochondrial genes are inherited only from the mother. The rest of the enzymes operating in the mitochondria are coded by nuclear DNA, synthesized on the rough endoplasmic reticulum and then are imported into existing mitochondria.

All cells in the mammalian body, with the exception of the erythrocyte, possess mitochondria. The erythrocyte lose their mitochondria during erythropoiesis at phase 3 (maturation) where normoblast eject the nucleus and organelle (Fig. **2.18**). Functional erythrocytes produce energy by fermentation through anaerobic glycolysis. The only and first mysterious eukaryote, oxymonad monocercomonoides species, was found without mitochondria [38].

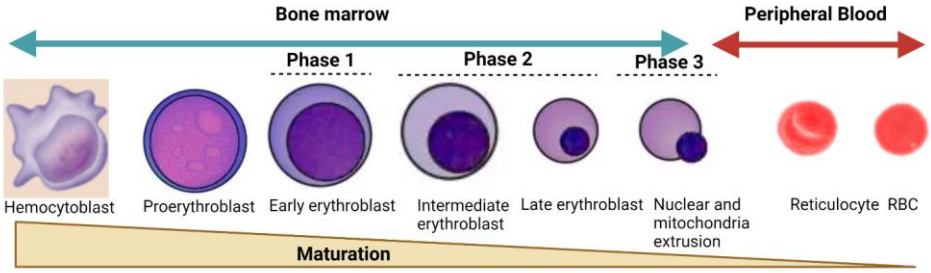


**Fig. (2.18). Schematic illustration of erythropoesis.** Erythropoesis starts with a haematopoietic stem cell, which differentiates into a common myeloid progenitor and then into a megakaryocyte-erythroid progenitor. Further differentiation results in a commitment to the erythroid lineage. The proerythroblast is the earliest morphologically identifiable bone marrow red cell precursor, characterized by a large cell with a high nuclear/cytoplasmic ratio, prominent nucleoli and blue cytoplasm (presence of RNA). As the erythroid cells mature, they become smaller, have more condensed chromatin, lose their nucleoli, and their cytoplasm changes from blue to pink. The nucleus and the organelle (mitochondria) are extruded, resulting in a reticulocyte. Most of the process takes place in the bone marrow over three weeks. The resulting reticulocyte retains some ribosomal RNA to make haemoglobin and after 1-2 days in the bone marrow, it enters the peripheral blood where the RNA is lost after 1-2 days resulting in a mature RBC. RBC, red blood cell.

### *2.1.3.4. Endoplasmic Reticulum*

The endoplasmic reticulum (ER) is an extensive network of branching and anastomosing membrane-limited channels and sacs (cisternae and flattened vesicles). The ER membrane divides the cytoplasm into two phases: the intracisternal (luminal) phase and the hyaloplasmic (cytosol) phase.

The ER is classified as either rough (granular) or smooth (agranular). Rough ER is studded with ribosomes. These ribosomes are distributed either along the cytosol or free in the cytosol and thereby named “attached” or “free” ribosomes, respectively. Ribosomes associated with the rough ER are mainly composed of ribosomal RNA and structural proteins and they are primarily involved in protein synthesis, a subject covered at length in chapter 5. Smooth ER, which is continuous with rough ER, however, does not have attached ribosomes and synthesizes phospholipids found in cell membranes as well as those that perform various other functions. In fact, smooth ER in the testes, for example, produces testosterone. In the liver, smooth ER plays an important role, through the cytochrome P450 and mixed-function oxidase system, in the detoxification and metabolism of many different drugs. In muscle cells, the smooth ER stores calcium ions. Certain portions of the ER may be continuous with the plasma membrane and the nuclear envelope. Regardless of any specialized function, the smooth ER also forms vesicles in which products are transported to the Golgi apparatus.

### *2.1.3.5. Golgi Apparatus*

The Golgi apparatus (also known as Golgi body or Golgi complex) was named for the Italian neuroscientist and biologist Camillo Golgi (1843-1926), who discovered its presence in Cell in 1898 and who was given the Nobel Prize in Physiology and Medicine in 1906 in recognition of his work on the nervous system structure.

The Golgi apparatus consists of a set of membrane-enclosed, smooth, and flattened cisternae stacked in parallel. It functions closely with the ER in the trafficking and sorting of proteins synthesized in the Cell. Three tubular networks at either end of the Golgi stacks have been identified [39]: the *cis*-Golgi network, which acts as an acceptor compartment of newly synthesized proteins coming from the ER, the *trans*-Golgi network, which is responsible for sorting proteins for delivery to their next destination [40], and the medial cisternae which are positioned between the *cis*- and *trans*-Golgi.

The Golgi apparatus receives proteins and lipid-filled vesicles that bud from the ER. The vesicle transport from the ER to the cis-Golgi complex, *via* the medial Golgi stacks, and from the trans-Golgi network to the plasma membrane is specifically controlled by specific proteins that serve as tags to tell the Golgi complex whether they are destined for secretion, inclusion in the plasma membrane, or incorporation in lysosomes.

### *2.1.3.6. Lysosomes*

Lysosomes were discovered by the 1974 Nobel Prize-winner Christian René Marie Joseph, Viscount de Duve (1917-2013), a Belgian cytologist and biochemist. They are membrane-bound vesicles produced and budded in the cytoplasm by the Golgi apparatus with the digestive enzymes (acid hydrolases) inside. Approximately 36 powerful enzymes, including proteases, glycosidases, and sulfatases capable of breaking down biomolecules like proteins, nucleic acids, carbohydrates, and lipids from the cell surface presented *via* endocytosis. Lysosomes also digest foreign materials such as viruses and bacteria during phagocytosis. Another catabolic activity of lysosomes is autolysis or autophagy, in which intracellular components including damaged organelles are digested as a result of dysfunction, degeneration or cell injury. Lysosomes are particularly large and abundant in macrophages and leukocytes, and they are humorously called “suicide sacs”.

Lysosomes play a key role in maintaining cellular homeostasis, plasma membrane repair, cell signaling [41], and energy metabolism. For instance, lysosomes of the osteoblasts promote the dissolution of minerals and the digestion of collagen, both

of which actions are necessary in bone resorption/remodeling and regulation of calcium and phosphorus homeostasis [42]. In addition, lysosomes are involved in the regulation of hormone secretion, such as that of thyroid hormones [43]. Malfunction of lysosomes can result in lysosomal storage diseases such as Tay-Sachs and Pompe's diseases.

### ***2.1.3.7. Peroxisomes***

Similar to lysosomes, peroxisomes are also discovered by Christian René Marie Joseph, Viscount de Duve, and are membrane-bound vesicles or microbodies that also contain a variety of enzymes. However and in contrast to lysosomes which are formed in the Golgi complex, peroxisomes self-replicate, and their enzymes are synthesized by cytoplasmic ribosomes and transported into peroxisome by carrier proteins. The primary function of these enzymes is to rid the Cell of toxic substances, and in particular, hydrogen peroxide ($H_2O_2$) by the catalase enzyme [44]. Indeed, Catalase converts the potentially harmful $H_2O_2$ to water and oxygen. Some types of peroxisomes, such as those in liver cells, detoxify alcohol (ethanol) *via* oxidation by alcohol dehydrogenase and microsomal cytochrome P-450 systems. The ethanol is oxidatively degraded to acetaldehyde and then to acetate. The acetate is subsequently converted to acetyl-CoA, transported to the mitochondria for oxidation *via* the Krebs cycle (chapter 6). Absence or reduced number of peroxisomes in the Cell has been shown to be linked to Zellweger syndrome, which usually causes death within the first year of life and for which there is no effective treatment [45, 46].

### ***2.1.3.8. Autophagosomes***

Autophagosomes are spherical structures with double-layer membranes. They are key structures in macroautophagy, the evolutionary conserved intracellular degradation process for cytoplasmic contents (damaged organelles, *etc*.) and also for invading microorganisms. Several autophagy-related (Atg) genes orchestrate the different sequential steps of autophagosome formation. The first two steps, initiation (step 1) and nucleation (step 2), involve the recruitment of cytosolic components of the core autophagic Atg1 kinase complex to the omegasomes [47]. The third step is the expansion and elongation of the double-membrane phagophores [48]. The phagophores surround, engulf, and entrap the cargo designated for autophagy and are close to forming mature autophagosomes (step 4) [49]. The autophagosomes fuse either directly to lysosomes to form autophagolysosomes or to late endosomes to give amphisomes that subsequently fuse with lysosomes resulting in degradation of the contents [50].

Autophagy machinery plays a pivotal role in maintaining cellular metabolism and homeostasis and thereby participates in a plethora of (patho)physiological

processes ranging from starvation adaptation, intracellular protein and organelle clearance, cell differentiation and development, innate and adaptive immunity, tumor suppression, and lifespan extension [51, 52]. Autophagy dysregulation is linked with various diseases such as cancer, metabolic syndrome, cardiomyopathy, muscular disorders, and neurodegeneration

### *2.1.3.9. Ribosomes*

Ribosomes are first described by the Romanian-American cell biologist George Emil Palade who was awarded the Nobel Prize in Physiology and Medicine along with Christian de Duve in 1974. Ribosomes are present in large numbers in all living cells and serve as the site of protein synthesis (chapter 5). They can be free or attached to the membrane of the ER and a single actively replicating eukaryotic cell may contain as many as 10 million ribosomes. The size of the ribosomes within cells varies depending on the cell type and physiological status (resting or replicating) of the Cell.

Ribosomes are made up of about 40% ribosomal proteins and 60% ribosomal RNA (rRNA) by weight. Based on their relative size, each ribosome is composed of two subunits, a large one called the 60S containing three rRNA species (5S, 5.8S, and 28S), and a smaller one designated 40S (18S) and 50 or more proteins. The subunits typically are referred to in terms of their sedimentation rate which is measured in Svedberg units (S). Proteins, synthesized at ribosomes attached to the endoplasmic reticulum have a different destination from that of proteins synthesized at ribosomes free in the cytoplasm.

### *2.1.3.10. Centrosome*

The centrosome was discovered by Edouard Van Beneden in 1883 and was described and named in 1988 by Theodor Boveri. As its Greek name indicates (*centrum* “center” and *Soma* “body”), the centrosome is an organelle that serves as the main microtubule-organizing center of the animal cell as well as a regulator of cell-cycle progression.

Centrosomes contain two centrioles arranged at right-angles to each other and are surrounded by the pericentriolar material (proteins) such as γ-tubulin, pericentrin, and mine in, which are responsible for microtubule nucleation and anchoring [53]. In many cell types, the centrosome is replaced by a cilium during cellular differentiation. Once the Cell starts to divide, the cilium is however replaced again by the centrosome [54]. During the prophase in the process of cell division, the centrosomes migrate to opposite poles of the Cell and interact with the chromosomes to build the mitotic spindle. Upon division, each daughter cell receives one centrosome.

### *2.1.3.11. Flagella and Cilia*

In eukaryotic cells, cilia are much shorter than flagella, but they have similar construction. Both are membrane-bound cylinders. The cylinders are composed of nine microtubule doublets arranged in a circle around two central microtubules. In contrast to protozoa and other microorganisms where cilia and flagella are used for locomotion, in eukaryotic cells, these organelles are employed to move substrate across the cell surface such as mucus in the respiratory tract.

### *2.1.3.12. The Cytoskeleton*

The cytoskeleton consists of arrays of thin filaments, intermediate filaments, thick filaments, and microtubules. These structures give shape and form to the Cell and are also involved in the movement of the Cell and its organelles. However, the cytoskeleton is dynamic because its protein components can assemble and disassemble as needed.

## CONCLUSION

At the lowest level of the hierarchy of an organismal biological structure, cells (the smallest units of life) are organized into tissues that carry out a specific task. Tissues are arranged together to form organs that execute particular functions, and group of organs with related functions compose the organ systems.

Organisms need an outside source of materials and energy to maintain their homeostatic organization and perform other life's activities. This energy is acquired by eating food. The consumed food passes through fascinating processes from ingestion to excretion, where it is mechanically and chemically broken down to provide the necessary nutrients and energy for the cells.

## REFERENCES

[1] Bowler PJ. Preformation and pre-existence in the seventeenth century: A brief analysis. J Hist Biol 1971; 4(2): 221-44.
[http://dx.doi.org/10.1007/BF00138311] [PMID: 11609422]

[2] Singer BR. Robert Hooke on memory, association and time perception (I). Notes Rec R Soc Lond 1976; 31(1): 115-31.
[http://dx.doi.org/10.1098/rsnr.1976.0003] [PMID: 11609928]

[3] Nezelof C. Henri dutrochet (1776–1847): an unheralded discoverer of the cell. Ann Diagn Pathol 2003; 7(4): 264-72.
[http://dx.doi.org/10.1016/S1092-9134(03)00075-3] [PMID: 12913852]

[4] Harris H. The birth of the Cell Y U Press, Ed. 1999.

[5] Gershon MD, Erde SM. The nervous system of the gut. Gastroenterology 1981; 80(6): 1571-94.
[http://dx.doi.org/10.1016/0016-5085(81)90275-4] [PMID: 6112192]

[6] Gershon M D. The enteric nervous system: a second brain. Hosp Pract 1999. 34, 31-32, 35-38, 41-32

passim

[7] Cooke HJ. Role of the "little brain" in the gut in water and electrolyte homeostasis. FASEB J 1989; 3(2): 127-38.
[http://dx.doi.org/10.1096/fasebj.3.2.2464517] [PMID: 2464517]

[8] Cooper MD, Peterson RDA, Good RA. Delineation of the Thymic and Bursal Lymphoid Systems in the Chicken. Nature 1965; 205(4967): 143-6.
[http://dx.doi.org/10.1038/205143a0] [PMID: 14276257]

[9] Schwarz BA, Bhandoola A. Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. Immunol Rev 2006; 209(1): 47-57.
[http://dx.doi.org/10.1111/j.0105-2896.2006.00350.x] [PMID: 16448533]

[10] Karasawa Y, Tasaki I, Yokota HO, Shibata F. Comparative effect of intravenously administered nitrogenous compounds on uric acid synthesis in chickens fed a 20 per cent protein diet. J Nutr 1973; 103(8): 1208-11.
[http://dx.doi.org/10.1093/jn/103.8.1208] [PMID: 4719730]

[11] Ellegren H. Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. Nat Rev Genet 2011; 12(3): 157-66.
[http://dx.doi.org/10.1038/nrg2948] [PMID: 21301475]

[12] Grützner F, Rens W, Tsend-Ayush E, *et al.* In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. Nature 2004; 432(7019): 913-7.
[http://dx.doi.org/10.1038/nature03021] [PMID: 15502814]

[13] Sakurai T, *et al.* Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior 1998. Cell 92, 1 page following 696
[http://dx.doi.org/10.1016/S0092-8674(00)80949-6]

[14] Labella FS, Beaulieu G, Reiffenstein RJ. Evidence for the existence of separate vasopressin and oxytocin containing granules in the neurohypophysis. Nature 1962; 193(4811): 173-4.
[http://dx.doi.org/10.1038/193173a0] [PMID: 14461249]

[15] Samuni L, Preis A, Mundry R, Deschner T, Crockford C, Wittig RM. Oxytocin reactivity during intergroup conflict in wild chimpanzees. Proc Natl Acad Sci USA 2017; 114(2): 268-73.
[http://dx.doi.org/10.1073/pnas.1616812114] [PMID: 28028227]

[16] Begg DJ, Munro HN. Action of thyroid-stimulating hormone on ribonucleic acid synthesis in thyroid slices and in isolated thyroid nuclei. Nature 1965; 207(4996): 483-5.
[http://dx.doi.org/10.1038/207483a0] [PMID: 5886132]

[17] Mittra I. Mammotropic effect of prolactin enhanced by thyroidectomy. Nature 1974; 248(5448): 525-6.
[http://dx.doi.org/10.1038/248525a0] [PMID: 4824351]

[18] Eppig JJ. FSH stimulates hyaluronic acid synthesis by oocyte–cumulus cell complexes from mouse preovulatory follicles. Nature 1979; 281(5731): 483-4.
[http://dx.doi.org/10.1038/281483a0] [PMID: 492307]

[19] Moudgal RP, Razdan MN. Induction of ovulation *in vitro* by LH and catecholamines in hens is mediated by α-adrenergic receptors. Nature 1981; 293(5835): 738-9.
[http://dx.doi.org/10.1038/293738a0] [PMID: 6270572]

[20] Dixon HBF, Stack-Dunne MP, Young FG, Cater DB. Influence of adrenotropic hormone fractions on adrenal repair and on adrenal ascorbic acid. Nature 1951; 168(4286): 1084-5.
[http://dx.doi.org/10.1038/1681084a0] [PMID: 14910647]

[21] Clarke DW, Wrenshall GA, Mayer J. Effects of pituitary growth hormone on the insulin and hyperglycaemic-glycogenolytic factor extractable from the pancreas of obese-hyperglycaemic mice. Nature 1956; 177(4522): 1235.
[http://dx.doi.org/10.1038/1771235a0] [PMID: 13334517]

[22] Yates CA, Herbert J. Differential circadian rhythms in pineal and hypothalamic 5-HT induced by artificial photoperiods or melatonin. Nature 1976; 262(5565): 219-20.
[http://dx.doi.org/10.1038/262219a0] [PMID: 934338]

[23] Smith R. Thermogenesis and Thyroid Action. Nature 1964; 204(4965): 1311-2.
[http://dx.doi.org/10.1038/2041311a0] [PMID: 14254423]

[24] Parsons JA, Robinson CJ. Calcium shift into bone causing transient hypocalcaemia after injection of parathyroid hormone. Nature 1971; 230(5296): 581-2.
[http://dx.doi.org/10.1038/230581a0] [PMID: 4928666]

[25] Mutchnick MG, Prieto JA, Schaffner JA, Weller FE. Thymosin modulation of regulatory T cell function. Clin Immunol Immunopathol 1982; 23(3): 626-33.
[http://dx.doi.org/10.1016/0090-1229(82)90325-7] [PMID: 6214350]

[26] Leupin E, Verzár F. Influence of desoxycorticosterone on glycogen formation and glucose uptake of isolated muscle. Nature 1949; 163(4152): 836.
[http://dx.doi.org/10.1038/163836a0] [PMID: 18144278]

[27] Ross EJ. Insulin and the permeability of cell membranes to glucose. Nature 1953; 171(4342): 125.
[http://dx.doi.org/10.1038/171125a0] [PMID: 13025503]

[28] Meistrich ML, Hughes TJ, Bruce WR. Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone. Nature 1975; 258(5531): 145-7.
[http://dx.doi.org/10.1038/258145a0] [PMID: 1186893]

[29] Schönberg A, Ghoneim A. Influence of different synthetic oestrogenic compounds on the egg-laying capacity and the growth of poultry. Nature 1946; 157(3977): 77-8.
[http://dx.doi.org/10.1038/157077b0] [PMID: 21065847]

[30] Schroeder LR, Gurney CW, Wackman N. Assay of erythropoietin in bone marrow suspensions. Nature 1958; 181(4622): 1537-8.
[http://dx.doi.org/10.1038/1811537b0] [PMID: 13566057]

[31] Campbell DJ, Bouhnik J, Ménard J, Corvol P. Identity of angiotensinogen precursors of rat brain and liver. Nature 1984; 308(5955): 206-8.
[http://dx.doi.org/10.1038/308206a0] [PMID: 6199679]

[32] Baugh CM, Barcena J, Bravo J, Dragstedt LR. Studies on the site and mechanism of gastrin release. Surg Forum 1957; 7: 356-60.
[PMID: 13433387]

[33] Houpt TR, Anika SM, Wolff NC. Satiety effects of cholecystokinin and caerulein in rabbits. Am J Physiol 1978; 235(1): R23-8.
[PMID: 677336]

[34] Pelleymounter MA, Cullen MJ, Baker MB, *et al.* Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995; 269(5223): 540-3.
[http://dx.doi.org/10.1126/science.7624776] [PMID: 7624776]

[35] Fukuhara A, Matsuda M, Nishizawa M, *et al.* Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005; 307(5708): 426-30.
[http://dx.doi.org/10.1126/science.1097243] [PMID: 15604363]

[36] Masuzaki H, Ogawa Y, Sagawa N, *et al.* Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. Nat Med 1997; 3(9): 1029-33.
[http://dx.doi.org/10.1038/nm0997-1029] [PMID: 9288733]

[37] Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. Nat Rev Genet 2012; 13(12): 878-90.
[http://dx.doi.org/10.1038/nrg3275] [PMID: 23154810]

[38] Karnkowska A, Vacek V, Zubáčová Z, *et al.* A Eukaryote without a Mitochondrial Organelle. Curr

Biol 2016; 26(10): 1274-84.
[http://dx.doi.org/10.1016/j.cub.2016.03.053] [PMID: 27185558]

[39] Rothman JE. The golgi apparatus: two organelles in tandem. Science 1981; 213(4513): 1212-9.
[http://dx.doi.org/10.1126/science.7268428] [PMID: 7268428]

[40] Griffiths G, Simons K. The trans Golgi network: sorting at the exit site of the Golgi complex. Science 1986; 234(4775): 438-43.
[http://dx.doi.org/10.1126/science.2945253] [PMID: 2945253]

[41] Vincent JP. Membranes, trafficking, and signaling during animal development. Cell 2003; 112(6): 745-9.
[http://dx.doi.org/10.1016/S0092-8674(03)00198-3] [PMID: 12654242]

[42] Lange PF, Wartosch L, Jentsch TJ, Fuhrmann JC. ClC-7 requires Ostm1 as a β-subunit to support bone resorption and lysosomal function. Nature 2006; 440(7081): 220-3.
[http://dx.doi.org/10.1038/nature04535] [PMID: 16525474]

[43] Marinò M, Zheng G, Chiovato L, *et al.* Role of megalin (gp330) in transcytosis of thyroglobulin by thyroid cells. A novel function in the control of thyroid hormone release. J Biol Chem 2000; 275(10): 7125-37.
[http://dx.doi.org/10.1074/jbc.275.10.7125] [PMID: 10702280]

[44] Herzog V, Fahimi HD. Microbodies (peroxisomes) containing catalase in myocardium: morphological and biochemical evidence. Science 1974; 185(4147): 271-3.
[http://dx.doi.org/10.1126/science.185.4147.271] [PMID: 4833829]

[45] Shimozawa N, Tsukamoto T, Suzuki Y, *et al.* A human gene responsible for Zellweger syndrome that affects peroxisome assembly. Science 1992; 255(5048): 1132-4.
[http://dx.doi.org/10.1126/science.1546315] [PMID: 1546315]

[46] Santos MJ, Imanaka T, Shio H, Small GM, Lazarow PB. Peroxisomal membrane ghosts in Zellweger syndrome--aberrant organelle assembly. Science 1988; 239(4847): 1536-8.
[http://dx.doi.org/10.1126/science.3281254] [PMID: 3281254]

[47] Cottam EM, Maier HJ, Manifava M, *et al.* Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum *via* an omegasome intermediate. Autophagy 2011; 7(11): 1335-47.
[http://dx.doi.org/10.4161/auto.7.11.16642] [PMID: 21799305]

[48] Polson HEJ, de Lartigue J, Rigden DJ, *et al.* Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy 2010; 6(4): 506-22.
[http://dx.doi.org/10.4161/auto.6.4.11863] [PMID: 20505359]

[49] Bodemann BO, Orvedahl A, Cheng T, *et al.* RalB and the exocyst mediate the cellular starvation response by direct activation of autophagosome assembly. Cell 2011; 144(2): 253-67.
[http://dx.doi.org/10.1016/j.cell.2010.12.018] [PMID: 21241894]

[50] Diao J, Liu R, Rong Y, *et al.* ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. Nature 2015; 520(7548): 563-6.
[http://dx.doi.org/10.1038/nature14147] [PMID: 25686604]

[51] Cinque L, Forrester A, Bartolomeo R, *et al.* FGF signalling regulates bone growth through autophagy. Nature 2015; 528(7581): 272-5.
[http://dx.doi.org/10.1038/nature16063] [PMID: 26595272]

[52] García-Prat L, Martínez-Vicente M, Perdiguero E, *et al.* Autophagy maintains stemness by preventing senescence. Nature 2016; 529(7584): 37-42.
[http://dx.doi.org/10.1038/nature16187] [PMID: 26738589]

[53] Eddé B, Rossier J, Le Caer JP, Desbruyères E, Gros F, Denoulet P. Posttranslational Glutamylation of α-tubulin. Science 1990; 247(4938): 83-5.
[http://dx.doi.org/10.1126/science.1967194] [PMID: 1967194]

[54] Avidor-Reiss T, Gopalakrishnan J. Cell cycle regulation of the centrosome and cilium. Drug Discov Today Dis Mech 2013; 10(3-4): e119-24.
[http://dx.doi.org/10.1016/j.ddmec.2013.03.002] [PMID: 24982683]

# CHAPTER 3

# Food Intake Regulation: Factors Influencing Food Intake

**Abstract:** Understanding the regulation of food intake is critical to managing body weight, health, growth, and metabolic disorders. Eating behavior is controlled by various factors and signals. This chapter aims to define and discuss the external factors, such as chemical senses, flavor, taste, aroma, texture, sight of food, and a variety of environmental, social and psychological that regulate appetite and feeding behavior.

**Keywords:** Appetite, Cultural factors, Environmental factors, Feeding behavior, Food choice, (Psycho)-socioeconomic factors, Physiological factors.

## INTRODUCTION

As I stated in Chapter 1, we eat because we are hungry and drink because we are thirsty. Before I discuss the chemistry and biochemistry determining the fate of food and water in the body, I will describe, in this chapter, the complex physiological and psychological mechanisms involved in the control of not only hunger, thirst, and satiety but also the appetite for different types of food. I will also uncover the relationship between food intake, energy expenditure, and body weight and close the chapter by describing some of the known disorders of appetite.

### 3.1. Regulation of Feed Intake

The first two questions that one might ask are: 1) Is there a “set point” for human and animal body weight? And 2) in comparison to humans, is feed intake in animals tightly controlled or random and unplanned?

The set-point theory postulates that human body weight is regulated at a predetermined level and is maintained at a relatively stable level for long periods. This regulation involves a feedback mechanism between the periphery and the CNS to correct any deviations in body weight from set-point. Evidence indicates that many factors, including nutrients, dietary composition, hormones, neural

pathways, behavioral, and physiological mechanisms, are involved to "defend" a certain range of body weight [1 - 3].

The best evidence for this is that when people choose a diet to eat less, most of them can lose some weight. However, throughout time, the body weight returns to about the same level. Moreover, when people lose weight following fat tissue removal by lipectomy or liposuction, they gain body weight through an increase in their food intake, which, in turn, allows body fat to slide back to its former, pre-operational level.

Similar to humans, large farm animals do have body weight set-point [4]. Birds are also able to adjust their feed intake according to their metabolisable energy, protein, and/or carbohydrate requirement. Indeed, Plavnik and Hurwitz showed, in the 1990's, that chickens (*gallus gallus domesticus)* and turkeys (*meleagris gallopavo*) were able to return to their initial body weight after feed restriction followed by *ad libitum* re-feeding [5]. It has also been reported that force-fed birds were able to return to their initial body weight when they had free access to food [6].

For both humans and animals, the set-point for bodyweight varies among individuals and sometimes in a time-dependent manner, with some remaining lean throughout their lives while others stand at a normal weight or in the overweight (obesity) range. This is due mainly to genetic determinants and their interactions with environmental factors. The individual has no control over some of these factors, including developmental determinants, genetic makeup, gender, and age. Other factors that influence body weight over which the individual has potential government encompass a level of physical activity, diet, and some environmental and social factors.

Interestingly, the animal genetic selection for high growth rate and high phenotypic feed efficiency, which is defined as the ability of an animal to convert feed nutrients into products; milk, meat, egg, *etc*., has markedly improved livestock productivity over the past 70 years. However, associated with these successes, there have been a number of unwanted changes in the regulation of feed intake. For instance, modern broilers (meat-type) chickens became hyperphagic[1] and achieved a 100-fold increase in body weight arising mainly from pectoralis (breast) muscle and abdominal fat during a period of 56 days [7, 8]. This indicates that modern broilers might lose their body weight set-point due to changes in the complex molecular mechanisms regulating feed intake, energy expenditure, and/or intermediary metabolism related to nutrient utilization and partition.

### *3.1.1. Factors that Influence the Choice of Food*

#### *3.1.1.1. Food Intake and Chemical Senses*

Human beings have developed and elaborated complex, overlapping, and sometimes redundant physiological mechanisms to ensure that the needs of the body for metabolic fuels, nutrients, and energy are met and the energy homeostasis[2] (relative stable equilibrium), which is the balance between feed intake and energy expenditure, is maintained.

One of the key components that controls feed intake is appetite, which is related not only to physiological need (hunger) but also to the pleasure of eating-flavor, taste, aroma, texture, sight of food, and a variety of social and psychological factors. In fact, the sensory properties of food is largely, if not primarily, determinant of food choice [9].

##### *3.1.1.1.1. Gustation*

In mammals, the peripheral gustatory system is composed of specialized epithelial cells located on the tongue, soft palate, pharynx, epiglottis, larynx, and upper third of the esophagus. The tongue taste buds (5,000 average number), which contain sensory cells with microvilli, can distinguish five basic tastes: salt, sweet, bitter, sour, and umami (the Japanese for savory). An additional taste mechanism for dietary fat with particular sensitivity for essential fatty acids has been postulated [10].

There is evidence that the sensation of savor is largely due to the presence of free amino acids in foods, and permits detection and selection of protein-rich foods. Sensitivity to sweetness aids carbohydrate detection and consumption, the salt taste is related to electrolyte (mainly mineral sodium) balance, sourness facilitates avoidance of strong acids, and bitterness enables toxin detection and rejection. Increasing evidence indicates that chemosensory inputs aid in correcting some specific nutrient imbalances such as calcium [11] and amino acids [12].

It has been reported that 25% of the population are non-tasters, and 75% are tasters. This differential taste ability was reported in 1931 by Arthur L. Fox, who discovered that some individuals found phenylthiocarbamide (PTC) to be bitter, however others found it tasteless [13]. Subsequent work conducted by Linda Bartoshuk and colleagues revealed that the taster group could be further divided into medium tasters and supertasters [14]. This heightened response was thought to be related to the presence of bitter-taste receptor genes TAS2Rs, which are G protein-coupled receptors expressed on the microvilli of taste receptor cells

located in the taste buds on the tongue [15] (Fig. **3.1a-c**). Supertasters tend to have more fungiform papillae and pain receptors than medium tasters and non-tasters [16] (Fig. **3.1b**). The perception of bitter taste is thought to have evolved as a protective mechanism for the avoidance of toxic substances, mainly those produced by plants [17]. The expression of RAS2Rs outside of taste buds suggests that these receptors might have other functions beyond bitter taste perception. For instance, TAS2Rs are expressed in rodent nasal epithelium on solitary chemosensory cells and thereby can detect airborne irritants and trigger a protective respiratory response [18]. Shah *et al*. [19] showed that TAS2Rs are also expressed on the motile cilia of human airway epithelial cells, suggesting a potential defensive response that aids in eliminating inhaled noxious compounds. Sternini *et al*. [20], on the other hand, found TAS2Rs to be expressed in the gut enteroendocrine cells and hypothesized that TAS2Rs might invoke protective responses such as vomiting after ingestion of harmful substances.

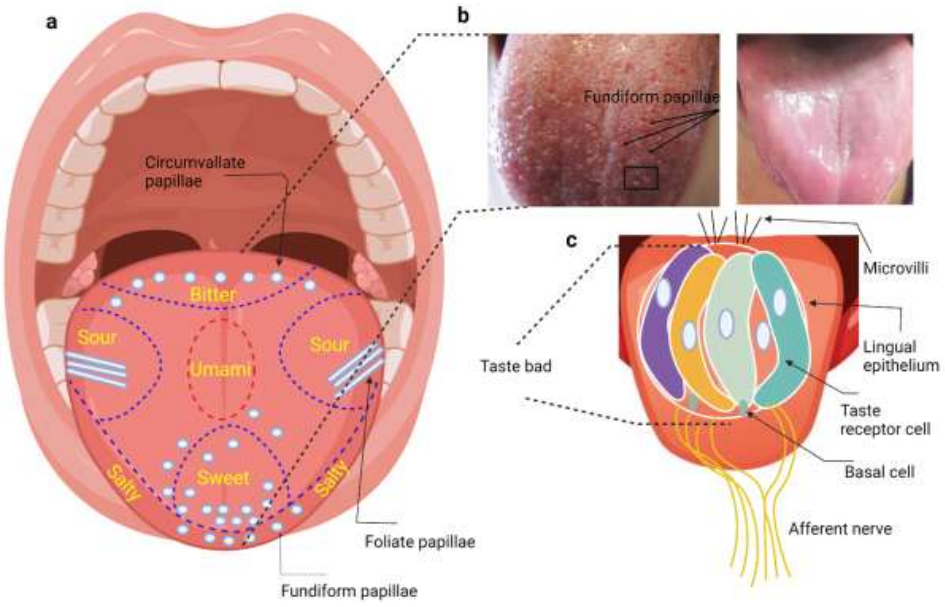


**Fig. (3.1). Taste area distribution (not for scale) and taste bud anatomy**. **(a)** Schematic representations of the taste (sweet, salty, sour, bitter, and umami) are on a human tongue. **(b)** Comparison of supertaster- and non-taster-tongue. **(c)** Diagram of the anatomical structure of a taste bud on a tongue. The figure was made using Biorender.com.

Although birds, for example, have fewer taste receptors and taste receptor genes relative to other vertebrates, they have a well-developed system for gustation and

can use taste cues to select nutrients and avoid toxins [21]. TAS2R sequences have been described in chickens and white-throated sparrows (*Zonotrichia albicollis*) [22, 23]. Interestingly, the sweet taste receptor gene TAS1R2 is absent in all bird genomes sequenced thus far, yet birds are able to taste sweet. For instance, the sugar detection thresholds of broad-billed hummingbirds (*Cynanthus latirostris*) are between 1.54-1.75 mM and 1.31-1.54 mM for glucose and sucrose, respectively [24, 25]. The salt (sodium chloride, NaCl) rejection threshold varies from 0.35% in parrot to 37% in the pine siskin (*Carduelis pinus*). Birds are also able to detect sour and bitter as well as umami [26, 27].

#### *3.1.1.1.2. Olfaction*

In addition to the sensation of taste provided by the taste buds on the tongue, the flavor can be distinguished by the sense of smell. Volatile molecules from olfactory stimuli reach the olfactory epithelium, which contains about $10^7$ receptor cells and more than 900 genes coding for various types of receptors, and thereby detect more than 10,000 distinct odors. The smell of some flavors and aromas is pleasurable, stimulating appetite and tempting people to eat more. However, other smells are repulsive, warning people not to eat food. Intriguingly, sometimes a pleasant smell to one person might be repulsive to another. This variability in people's perception is probably due in large part to genetic variations in a single or several odorant receptors [28].

In domestic chickens, for example, the olfactory epithelium contains receptor cells with 6 to 15 7-10 μm cilia. By using the cardiac conditioning technique, Davis RG [29] has shown that most birds exhibit olfactory capabilities comparable to mammals. Interestingly, the European starlings (*Sturnus vulgaris*) can distinguish between plant odors during spring only (period of nest building), rather than in summer and fall, suggesting a hormonal milieu influence [30].

### ***3.1.1.2. (Psycho)socio-economic and Cultural Factors***

Among the key factor that determines the choice of food is the availability and the cost of food itself. For instance, in developed countries, the availability of food is not a limitation because there is a wide variety of abundant foods available, and if not, they are imported, frozen, canned and/or dried. By contrast, in developing countries, the availability of food is a major constraint. This (un)availability plays a major role in determining the cost of foods, and for the most disadvantaged members of the community, limited incomes may impose severe restrictions on their choice of foods.

Health status and age are two important factors affecting not only the choice but also the quantities of food consumed. Illness impairs appetite (see section 3.1.1.7), and some of the medicines (drugs) depress appetite through distortion of taste and/or by causing nausea.

Many lonely single people, especially the elderly, have little motivation to prepare food and less desire to eat, and even to carry food when they have health issues such as arthritis or osteomyelitis. If these conditions are combined with low incomes, this would severely limit the range of foods consumed and, in severe cases, can lead to undernutrition.

Conversely, humans are likely to eat more and better when they are in a group compared to people eating alone. This is due not only to the greater variety of dishes offered but also to the social facilitation or group stimulation/motivation [31, 32]. Similarly, animals have also developed the social facilitation of eating [33, 34]. In a previous study, we have administered an anorexigenic[3] compound (leptin, appetite inhibitor) in one group of chickens, while the control group was untreated, and we have monitored the feeding behavior of both groups. The treated birds ate less but displayed similar time-spent eating and number of access to the feeders as the control group, indicating a clear congener imitation and social motivation [35].

Regional, cultural, habit, tradition, ethical consideration, and religion are important factors in determining the choice of foods. For instance, observant Jews and Muslims eat only kosher meat from animals that have cloven hooves and chew the cud. Hindus, for example, on the other hand, do not eat beef. Other people refrain from eating meat due to humanitarian concerns for animals and the environment or because of real/perceived health benefits. Some avoid red meat because of the high levels of saturated fat, however others prefer red meat for the high levels of iron. Some people specifically avoid bovine, ovine, and /or deer meat because of the potential risk of transmissible spongiform encephalopathies (TSEs) such as bovine spongiform encephalopathy (BSE or mad cow disease), scrapie, and chronic wasting disease. Pescetarians eat fish, but no meat or poultry. Ovo-lacto-vegetarians eat eggs and milk, but no meat or fish. Lacto-vegetarians eat dairy but not eggs however, vegans eat only plant- but not animal-based foods.

Eating habits as adults continue the habits learned as children. My wife, for example, never eats green salad as a mixture of vegetable pieces, but she drinks it as juice. Some people are more adventurous than others and like to try new foods. Others are yet conservative, and they do not like new foods because they have never eaten them before. Many people choose to eat organically produced foods in preference to those produced by conventional or intensive farming methods.

### *3.1.1.3. Chemical and Physical Properties of the Food*

In human nutrition, a given food can exert specific satiety by limiting the intake of itself, or general satiety by inhibiting further consumption of other foods [36]. For instance, Porrini and co-workers have shown that baked macaroni and meat balls consumption had a higher satiety index than mixed boiled vegetables and fruit salad, which might be associated with the energy and protein content of the food [37]. Rolls and colleagues, on the other hand, using a pudding and a jello test meal varied in energy density, found that subjects ate a constant weight of food rather than a constant number of calories [38]. They further hypothesized that sensory-specific satiety, in terms of the changing hedonic[4] response, is associated with the sensory property of the food consumed rather than the macronutrient composition [36].

In livestock and particularly in poultry, food is a complex matrix in which the physical and chemical characteristics interact in different ways. Because it represents the highest cost in the production cycle [39] and it is primarily responsible for the growth performances, feedstuff is an aspect of high economic importance in commercial poultry production. Although the expensive manufacturing cost, using processed feed has been proven to be advantageous in commercial poultry production [40], however feed particle size and physical forms have been reported to differently affect feed intake. Indeed, Nir *et al*. [41] found greater feed intake and weight gain in the use of particles by roller mill because of the larger particle size and better uniformity of feed. As birds are able to select different sizes of feed particles, the uniformity of feed particles is very important for good performance. It has been suggested that at least 20-30% of the particle should present a size greater than 1000 μm because finely ground particles could inhibit the gizzard function [42]. Similarly, the moisture content of the feed has been reported to modulate feed intake and growth in broilers. In fact, Dei and Bumbie [43] have shown that broilers fed with wet mash exhibited higher feed intake and weight gain compared to those fed with dry mash in a hot climate.

### *3.1.1.4. Physiological Conditions of the Animal*

#### *3.1.1.4.1. Pregnancy-gestation*

Pregnancy and gestation are complex states where changes in maternal physiology have evolved to favor the development and growth of the placenta and the fetus. Along with these adaptations, women often make dietary changes, with generally decreased ingested food, although the reasons for these dietary modifications are not well defined. One potential common reason for reducing or

eliminating specific foods is the health of the baby, aversion, and/or nausea [44]. Women increased consumption of sweet foods more frequently than savory or spicy foods to satisfy the craving. This, in turn, may promote excess calorie intake and increased gestational weight gain [45].

In small ruminants (ewes and goats), the practice of increasing nutrient intake and body conditions during gestation is called steaming up. This is generally accomplished by providing ewes or goats with fresh pasture, supplemental forage, or grain on a daily basis. Flushing, however, is the practice of increasing nutrient intake and body condition prior to and during the breeding season. Its purpose is to increase the rate of ovulation. The response to both practices is influenced by: 1) age of the animal, 2) breed and strain, 3) initial body condition, 4) environmental condition and season (availability of forage), and 5) stage of the breeding season.

### *3.1.1.5. Management Factors*

Although a meal might mean something different to everyone, the psychologists Spence and Piqueras-Fiszman agreed and reported in their book "The perfect meal: the multisensory science of food and dining", that differential food arrangement and plating can enhance the dining experience and differentially stimulate appetite and eating behavior [46]. It has been reported that the shape and color of the dinnerware can affect the taste and food intake [47, 48]. For instance, round and white plates tend to enhance sweet flavors in food, while black and angular plates are likely to bring out more savory flavors [49]. Red plates, however, tend to reduce food intake [49, 50]. Although solid scientific evidence is not abundant, the hue of light/shade in the room may also affect food/drink perception and ingested quantities. For example, a strong coffee drinker would drink more under bright light, however, a weak coffee drinker tends to drink more under dim light. Rosenthal and colleagues have shown appetitive disturbances in seasonal affective disorder (SAD), which is a variant of clinical depression that is seasonal in nature and responsive to light therapy [51, 52]. Using rodents, studies showed a reduced appetite during constant light exposure [53], however, its relevance to human physiology is questionable as rodents are nocturnal feeders. Similarly, one environmental stimulus shown to impact food and fluid intake is music [54 - 56]. Distraction and modulation of mood states are some of the potential mechanisms by which music influences feeding and drinking behaviors [57, 58]. It has also been suggested that music can affect taste and flavor perception and thereby affect feeding and drinking behaviors [59 - 61].

As mentioned above, people enjoy meals more when they are eating with a group of friends than when they are eating alone. It is noteworthy to clarify here the

meaning of appetite, feed intake, and hunger because there is enormous variability in what individuals, including scientists, mean when they use them, and these terms are often used interchangeably and confusingly. Based on Wikipedia, appetite is the desire to eat food, whereas hunger usually refers to a more objective deprivation state, which is provoked by energy deficit. Although they are related (hunger often produces a desire of eating or appetite), someone may be hungry without feeling a desire to eat (case of anorexia nervosa[5], for example), or someone may desire to eat something even in the absence of a need for it. Food intake, the third appetite-related term, is the ingestion of any substance consisting of carbohydrates, proteins, fats, vitamins, and minerals. In contrast to animals, it is possible to distinguish in humans between what a person wants (desire and appetite), or needs (hunger), from what he/she actually eats.

In livestock, feeding behavior can be measured by feeding time, time-spent eating, meal frequency, feeding rate, and rumination time (for ruminants). It is affected by various hedonic[6], managerial, and motivational factors, including social interactions. Ginane *et al*. [62] have shown that social facilitation is a feeding motivation enhancer, in which an animal's motivation (whether that animal is hungry or not) to eat is stimulated by the sight and sound of other animals eating. In chickens, as shown in Fig. (**3.2**), a leptin-treated group manifested similar feeding behavior (time-spent eating and number of access to the feeders) as the control group, confirming the effect of social interaction and motivation. Designing and managing feeders and drinkers is, therefore, crucial and must consider social interaction and behavior [63]. Some authors consider that feeding management regulates eating behavior [64].

At the rearing environment, it is very critical that the food and water sources are properly arranged and well managed. Studies showed that design features such as size, location, geometry, spacing, and angle could affect the feeding and drinking behavior of the animal (for review see [65]). It is essential that the feeding and drinking equipment provide ease of access, reduce wastage, and avoid competition and crowd. The feeders and drinkers must be maintained at an optimal height. In fact, it has been reported that, at a lower height, the average body weight of chickens can be up to 7% higher [66], with a lower percentage of intramuscular fat in the thighs and greater muscle in drumsticks [67].

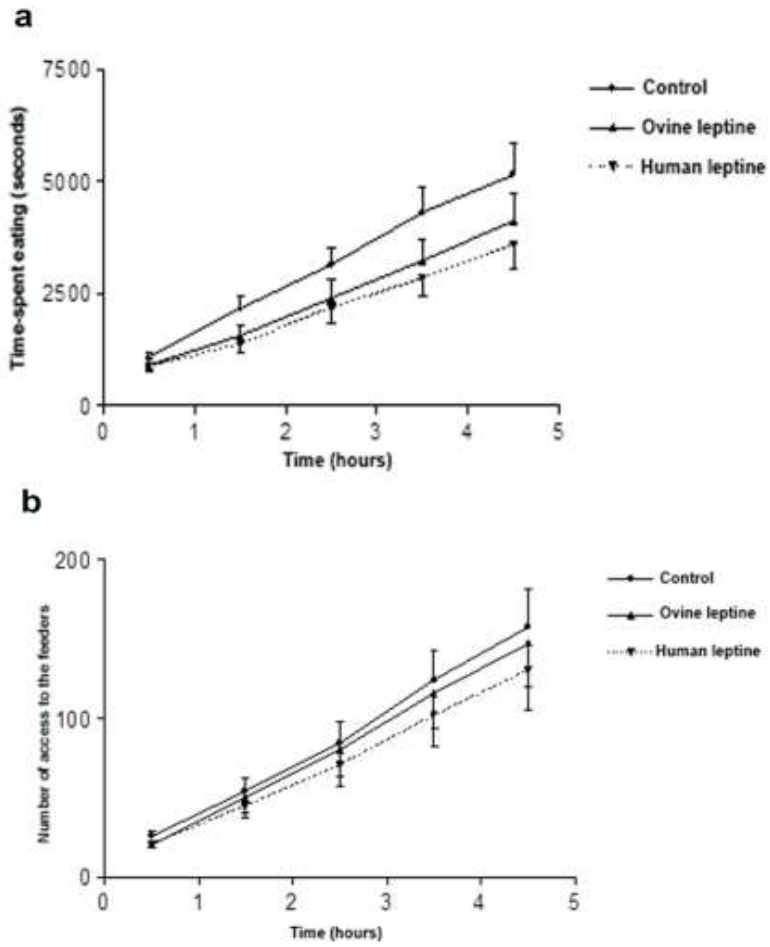


**Fig. (3.2). Social motivation and congener imitation in chickens**. Leptin (anorexigenic hormone)-treated chickens showed the same feeding behavior (time-spent eating and number of access to the feeders) as the placebo, confirming that social facilitation is a feeding motivation enhancer.

## *3.1.1.6. Environmental Factors*

The thermostatic theory of food intake, originally proposed by Brobeck in 1948

[68], is that the body experiences a temperature-dependent variation in energy needs that should be reflected in appetite and food intake. As the body core temperature raises through feeding (dietary-induced thermogenesis, also known as the thermic effect of food or specific dynamic action) in all endothermic (warm-blooded) animals, common knowledge and literature indicate that all these animals in laboratory or field setting eat less when the environmental temperature is high than when it is low. Body core and skin temperature as well as heart rate and blood volume and pressure, have been used as indices of the ability of the body to defend body temperature set point (thermoregulation[7]).

### *3.1.1.6.1. Heat Stress*

Heat stress occurs in animals when there is an imbalance between heat production within the body and its dissipation [69], and stress is generally defined as the magnitude of forces external to the body which tend to displace its system from its resting or ground state. Under heat load conditions, the body core temperature increases, and the thermoregulatory mechanisms involved in dissipating heat become fully operative. If normal food intake continues under these heat load conditions, the additional heat produced by ingested food may lead to hyperthermia. One of the earliest observations of the depressive effects of heat on appetite and food intake in a human was reported by Johnson and Kark in 1947 [70] and has been confirmed by succeeding studies [71 - 73]. In experimental models, Brobecks found that increased temperature above 32°C reduced food intake and body weight in rats [68]. Similar results have been extensively reported in other rodents [74 - 76]. In large farm animals, Hamzaoui *et al*. [77], have shown that goats eat less under hot ambient temperatures. Rhoads *et al*. [78] demonstrated that thermal stress also depressed feed intake in cows. Heat-stressed (32°-35°C) swine ate half as much as their counterparts maintained at 10° to 12°C [79, 80]. In modern poultry, which is particularly sensitive to high environmental temperature, the adverse effect of heat stress on feed intake is well known and extensively reported in many studies, including our own [81 - 87]. This reduced intake in the heat would thus seem to be adaptive mechanism to cope with hot environment. It is noteworthy to mention that the ability to defend one's body temperature against heat stress is influenced by level of activity, acclimatization state, aerobic fitness, and hydration level.

From a physiological standpoint, mechanisms for heat defense include thermoregulatory behavior to increase heat loss, cutaneous vasodilation to facilitate heat loss by conducting heat from the body core to the skin (body surface), and evaporative cooling *via* sweating, saliva spreading, or panting, employed to a various degree by different species [88]. From an anatomical

standpoint, however, the early results were confusing. For instance, Andersson and Larsson [89] showed similar responses as external cues to inhibit eating when heating the preoptic and anterior hypothalamus regions in animals. Spector *et al*. [90], however, showed a biphasic effect with increased eating when the temperature of the preoptic medialis region was raised to 43°C, and decreased intake when the area was heated to 35°C. The general consensus is that maintaining body temperature during environmental temperature challenges is orchestrated by thermoreceptors in primary sensory nerve endings distributed in the skin. These thermosensory signals are transmitted in a feedforward manner to a second-order thermal sensory neurons in the spinal and trigeminal dorsal horn, which project to third-order sensory neurons in the dorsal subnucleus of the lateral parabrachial nucleus (LPB) area. The thermosensory signals for thermoregulation responses are, then, transmitted from the LPB to the preoptic area which provide command signals descending to peripheral effectors and evoke behavioral, autonomic, somatic, and hormonal responses counteracting changes in environmental temperatures before they impact body core temperature [91 - 93].

As an example of non-mammalian species, chickens are endothermic[8] homeotherms[9] that regulate their body core temperature. As for mammals, avian thermoregulation is characterized by the maintenance of brain temperature below body core temperature, reflecting the high thermal sensitivity of brain tissues. It is important to mention that the body temperature in avians can differ from the skin temperature by several degrees. The mean deep body temperature measured in 202 species spanning most avian orders varied between 38.5°C and 41°C, and can reach up to 45°C under heat stress exposure. Heat can be transferred from bird's surface *via* radiation, convection, conduction, and evaporation. These processes will not be described in this chapter as they are out of the scope, but readers can find a detailed description in elegant reviews elsewhere [94].

The thermogenic response of avians and endotherms in general to changes in the environmental temperatures can be divided into three zones (Fig. **3.3**).

1. Comfort zone (thermoneutral zone) where body temperature is controlled mainly by conduction.
2. Zone of evaporative regulation where the body temperature is regulated by vasodilation, followed by evaporation and sensible heat loss.
3. Zone of body cooling where the body temperature is maintained by vasoconstriction for heat retention, followed by shivering[10] and non-shivering thermogenesis[11].

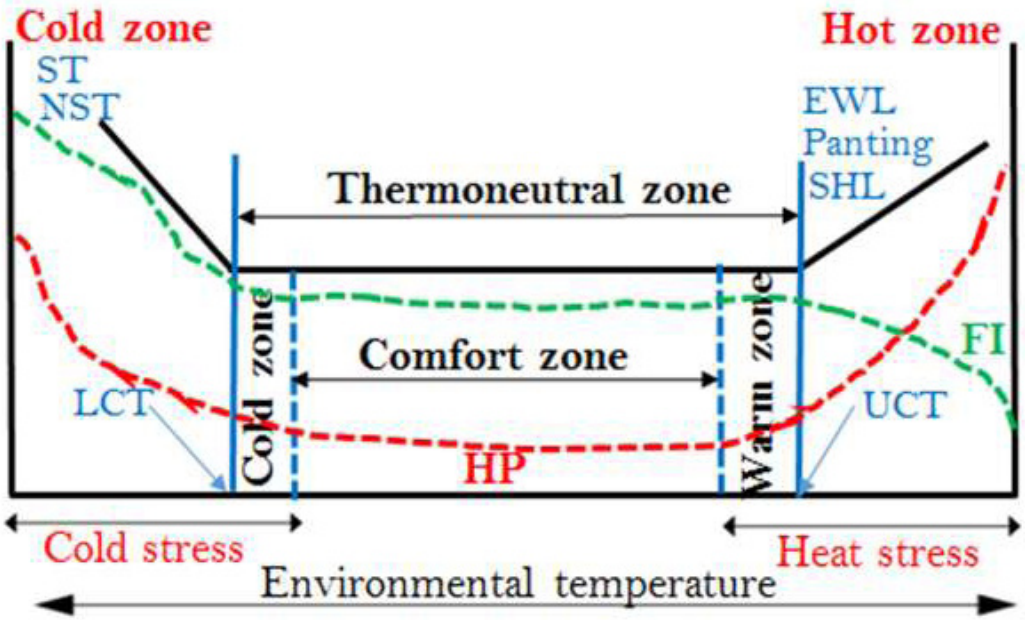


**Fig. (3.3). Thermogenic curve of endothermic organisms.** The thermoneutral and comfort zone ranged between the lower and upper critical temperatures. Below the LCT and under cold stress exposure, heat is generated by ST and NST and food intake is stimulated. Under heat stress conditions and above the UCT, thermogenesis increases to permit EWL mainly *via* panting and SHL. EWL, evaporative water loss; LCT, lower critical temperature; NST, non-shivering thermogenesis; SHL, sensible heat loss; ST, shivering thermogenesis; UCT, upper critical temperature.

*3.1.1.6.2. Dehydration*

One consequence related to heat stress is dehydration, which occurs with excess loss of total body water (sweating for example) and is often associated with electrolyte unbalance. There are three types of dehydration: 1) hypertonic dehydration, which occurs when more water than sodium is lost from the extracellular fluid compartment (inadequate water intake, sweating, osmotic laxatives), 2) hypotonic dehydration, which occurs when sodium loss is greater than water (vomiting, diarrhea, renal failure), and 3) isotonic dehydration, which results from a similar amount of water and sodium loss (ascites, vomiting).

It has been reported that decreased feed intake in non-acclimatized subjects in tropical climates may be mediated by hypertonicity associated with initial dehydration [95]. Furthermore, the administration of hypertonic solutions in rats resulted in decreased feed intake [96]. Dehydration has been shown to reduce feed intake in many species. For instance, Silanikove found that food consumption decreases with the lengthening of the dehydration period in goats [97]. Similar

effects were observed in white-tailed deer under water restriction (33%) conditions [98]. Water restriction has been shown to reduce feed intake and growth performance in chickens [99, 100]. In addition to reduced feed intake and growth performance, Arad [101] observed that, after 48 hours of water deprivation, dehydrated birds presented slightly higher body temperature when compared to normally hydrated birds. Dehydrated fowls were capable of recovering 92% of the initial body weight within 30 min of drinking. However, birds that lost more than 15% of their weight failed to recover it.

In humans, however, it is postulated that increased water consumption before and/or during meals may help obese individuals to lose body weight *via* reducing their food intake [102]. This regime seemed to work only for certain subjects [103, 104]. Recent studies indicated that the reducing effect of water on body weight is also associated with enhanced thermogenesis and energy expenditure [105 - 107].

*3.1.1.6.3. Cold Stress*

As for heat stress, cold exposure induces physiological changes. It is known that cold exposure increases appetite and energy intake in a wide range of animal species, but not in humans [68, 108, 109]. In contrast, LeBlanc reported that six studies conducted with military soldiers in arctic conditions showed an average body weight gain of 1 kg/month, which might be due to a change in appetite; however, these expeditions (studies) were not necessarily planned for research purposes [110]. Continuous cold exposure experiments in rodents have also yielded conflicting results, with some investigations showing declined body weight gain in rats and others showing no alteration in body weights [111 - 114]. In broiler (meat-type) chickens, Qureshi and colleagues showed that cumulative feed intake was significantly increased in birds reared under cold conditions compared to those maintained under thermoneutral conditions [115]. Baarendse *et al*. [116] reported that moderate cold exposure by reducing the environmental temperature by 1°C every day in five-day period, during the early post-hatch life caused long-term negative effects on chicken growth performance. Shinder *et al*. [117], on the other hand, showed that acute cold exposure at late embryogenesis improved growth performances. Dridi's group showed that chronic mild cold conditioning improved body weight and feed conversion ratio (FCR)[12] during the first-week post-hatch and at the market age [118]. These discrepancies might be due to various factors, including the experimental conditions (environmental temperature, exposure duration, severity and type), chicken strain and age, and diet composition. Independently of the effects on energy intake and expenditure, one might ask the question whether heat and cold stress produce differentially sensory responses and affect food choice.

*3.1.1.6.4. Effects of Environmental Factors (Heat or Cold) on Food Choice*

It appears that, in the summer or winter seasons, food choices do change, however, whether these changes are associated with the environmental temperature per se or to other factors such as availability and price have not been well established. Although Drewnowski and colleagues [119] reported that humans have an expressed preference for fats and sweets in temperate environments, there is still a dearth of solid experimental research on human food consumption preference under heat variations. Generally, intake of vegetables, fruits, and beverages increases in the summer however intake of legumes, sweets, meats, and eggs increases in the winter. Within each food subgroup, the consumption appeared to mirror the seasonal availability. For instance, more citrus fruits are eaten during winter, while apple intake is higher during summer. Intake of fats and oils increase in the winter and decline in the summer, except for the use in salad dressing.

It has been reported that cooling the tongue reduced the perceived intensity of the sucrose sweetness and the caffeine bitterness, but not that of sodium chloride saltiness and citric acid sourness [120], indicating that the temperature of the food itself affect the tastes of food. Trant and Pangborn [121] reported that warming certain food and beverages enhance their flavor and aroma.

*3.1.1.6.5. Effect of Light*

The evident effect of light on appetite and feed intake can be supported by the seasonal affective disorder (SAD) where the depressive phase is associated with overeating, carbohydrate craving, and body weight gain [51, 122]. Light therapy of SAD patients *via* periodic exposure to bright light (2500 lux full-spectrum fluorescent light) produced weight loss. There is still no scientific evidence that the appetite of normal individuals is affected by light exposure. In avian (non-mammalian) species, normothermic body temperature is characterized by a circadian rhythm, which is entrained by photoperiod and regulated by melatonin, suggesting that light regulates feeding behavior. Light (intensity, color, duration) inputs are perceived by the eye and pineal gland and reach the hypothalamic suprachiasmatic nucleus, with the melatonin hormone being the major signal. Melatonin has been reported to regulate food intake [123, 124]. Melatonin synthesis by gastrointestinal cells has also been suggested to be involved in adjusting body temperature in response to restricted food availability [125]. Photoperiod manipulation in broilers has largely consisted of maximizing feed intake [126]. Hester *et al*. [127] have shown that a low light intensity program resulted in more rapid and efficient growth at an older age.

However, Deep *et al*. [128] reported no effects of light intensity on feed consumption, body weight, and FCR.

### *3.1.1.7. Effects of Disease and Fever*

Several diseases such as cancer, viral, parasitic, or bacterial infection, endocrine and metabolic disorders, gastrointestinal disorders including Celiac disease, ulcers, and gastroesophageal reflux disease (GERD), psychiatric disorders (anxiety, depression, trauma, anorexia nervosa), cognitive disorders (dementia), organ failure can affect appetite and food intake. Some of the diseases are associated with fever, which is not an exogenous source of hyperthermia. Generally fever suppresses food intake [129]. However McCarthy *et al*. [130] showed that intraperitoneal injection of interleukin 1 (IL-1), pro-inflammatory cytokine, raised body temperature in rats and suppressed food intake, while IL-1 intracerebroventricular (ICV) administration raised body temperature without affecting energy intake. These data suggested that fever-induced anorexia may not be mediated by thermoregulatory mechanisms.

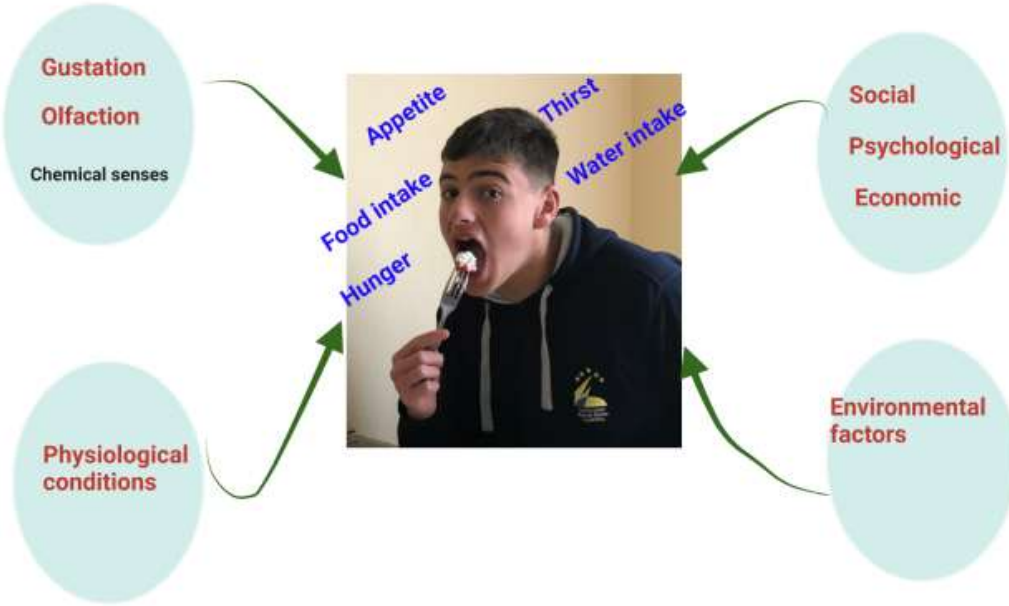


**Fig. (3.4). Factors influencing food choice and intake.** Appetite and feeding behavior are stimulated or inhibited by various external factors including the physical and chemical properties of the food itself as well as the environmental, cultural, psychological, sociological, and economic factors.

## CONCLUSION

Appetite and feeding behavior are stimulated or inhibited by various external factors, including the physical and chemical properties of the food itself as well as the environmental, cultural, psychological, sociological, and economic factors (Fig. **3.4**). However, there is also considerable variability related to the effects of the above-mentioned factors. For instance, some people may be particularly susceptible to one or more of these factors, while others may be resistant.

## NOTES

[1] Hyperphagia is an abnormally great desire for food and excessive eating

[2] Energy homeostasis is a biological process that involves the coordinated homeostatic regulation of energy inflow (feed intake) and outflow (energy expenditure).

[3] Anorexigenic or appetite inhibitor is a drug, hormone, or compound that blocks appetite and may induce hypophagia

[4] Hedonic is connected with feeling of pleasure and rewarding.

[5] Anorexia Nervosa is a life-threatening eating disorder that is characterized by self-starvation and excessive body weight loss

[6] Hedonic is connected with feeling of pleasure and rewarding.

[7] Thermoregulation: is the ability of an organism to maintain a core body temperature within an optimal physiological range and state of equilibrium.

[8] Endothermy: The pattern of thermoregulation of animals in which the body temperature depends on a high and controlled rate of heat production (based on IUPS Thermal Commission 2001).

[9] Homeothermy: is thermoregulation that maintains a stable internal body temperature regardless of external influence (Wikipedia).

[10] Shivering thermogenesis is a response to sudden exposure to cold and is a major contributor to enhanced heat production

[11] Nonshivering thermogenesis is defined as an increase in metabolic heat production (above the basal metabolism) that is not associated with muscle activity

[12] FCR: is the efficiency with which the body of livestock convert animal feed into desired output (meat, egg, milk, *etc*.)

## REFERENCES

[1] Harris RBS. Role of set□point theory in regulation of body weight. FASEB J 1990; 4(15): 3310-8.
[http://dx.doi.org/10.1096/fasebj.4.15.2253845] [PMID: 2253845]

[2] Wirtshafter D, Davis J. Set points, settling points, and the control of body weight. Physiol Behav 1977; 19(1): 75-8.
[http://dx.doi.org/10.1016/0031-9384(77)90162-7] [PMID: 11803695]

[3] Garrow JS, Stalley S. Is there a 'set point' for human body-weight? Proc Nutr Soc 1975; 34(2): 84A-5A.
[PMID: 1187645]

[4] Henry BA, Blache D, Dunshea FR, Clarke IJ. Altered "set-point" of the hypothalamus determines effects of cortisol on food intake, adiposity, and metabolic substrates in sheep. Domest Anim Endocrinol 2010; 38(1): 46-56.
[http://dx.doi.org/10.1016/j.domaniend.2009.07.006] [PMID: 19733031]

[5] Plavnik I, Hurwitz S. Response of broiler chickens and turkey poults to food restriction of varied severity during early life. Br Poult Sci 1991; 32(2): 343-52.
[http://dx.doi.org/10.1080/00071669108417359] [PMID: 1868373]

[6] Lepkovsky S. Hypothalamic-adipose tissue interrelationships. Fed Proc 1973; 32(6): 1705-8.
[PMID: 4710878]

[7] Scheuermann GN, Bilgili SF, Hess JB, Mulvaney DR. Breast muscle development in commercial broiler chickens. Poult Sci 2003; 82(10): 1648-58.
[http://dx.doi.org/10.1093/ps/82.10.1648] [PMID: 14601746]

[8] Hood RL. The cellular basis for growth of the abdominal fat pad in broiler-type chickens. Poult Sci 1982; 61(1): 117-21.
[http://dx.doi.org/10.3382/ps.0610117] [PMID: 7088775]

[9] Glanz K, Basil M, Maibach E, Goldberg J, Snyder D. Why Americans eat what they do: taste, nutrition, cost, convenience, and weight control concerns as influences on food consumption. J Am Diet Assoc 1998; 98(10): 1118-26.
[http://dx.doi.org/10.1016/S0002-8223(98)00260-0] [PMID: 9787717]

[10] Gilbertson TA, Fontenot DT, Liu L, Zhang H, Monroe WT. Fatty acid modulation of K+ channels in taste receptor cells: gustatory cues for dietary fat. Am J Physiol Cell Physiol 1997; 272(4): C1203-10.
[http://dx.doi.org/10.1152/ajpcell.1997.272.4.C1203] [PMID: 9142845]

[11] Leshem M, Del Canho S, Schulkin J. Calcium hunger in the parathyroidectomized rat is specific. Physiol Behav 1999; 67(4): 555-9.
[http://dx.doi.org/10.1016/S0031-9384(99)00113-4] [PMID: 10549893]

[12] Naito-Hoopes M, McArthur LH, Gietzen DW, Rogers QR. Learned preference and aversion for complete and isoleucine-devoid diets in rats. Physiol Behav 1993; 53(3): 485-94.
[http://dx.doi.org/10.1016/0031-9384(93)90142-3] [PMID: 8451313]

[13] Fox AF. Six in ten 'tasteblind' to bitter chemical. Sci News Lett 1931; 9

[14] Bartoshuk LM. Psychophysical advances aid the study of genetic variation in taste. Appetite 2000; 34(1): 105.
[http://dx.doi.org/10.1006/appe.1999.0287] [PMID: 10744897]

[15] Hayes JE, Bartoshuk LM, Kidd JR, Duffy VB. Supertasting and PROP bitterness depends on more than the TAS2R38 gene. Chem Senses 2008; 33(3): 255-65.
[http://dx.doi.org/10.1093/chemse/bjm084] [PMID: 18209019]

[16] Drewnowski A, Henderson SA, Barratt-Fornell A. Genetic taste markers and food preferences. Drug Metab Dispos 2001; 29(4 Pt 2): 535-8.
[PMID: 11259346]

[17] Glendinning JI. Is the bitter rejection response always adaptive? Physiol Behav 1994; 56(6): 1217-27.
[http://dx.doi.org/10.1016/0031-9384(94)90369-7] [PMID: 7878094]

[18] Finger TE, Böttger B, Hansen A, Anderson KT, Alimohammadi H, Silver WL. Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. Proc Natl Acad Sci USA 2003; 100(15): 8981-6.
[http://dx.doi.org/10.1073/pnas.1531172100] [PMID: 12857948]

[19] Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ. Motile cilia of human airway epithelia are chemosensory. Science 2009; 325(5944): 1131-4.
[http://dx.doi.org/10.1126/science.1173869] [PMID: 19628819]

[20] Sternini C, Anselmi L, Rozengurt E. Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. Curr Opin Endocrinol Diabetes Obes 2008; 15(1): 73-8.
[http://dx.doi.org/10.1097/MED.0b013e3282f43a73] [PMID: 18185066]

[21] Shi P, Zhang J. Contrasting modes of evolution between vertebrate sweet/umami receptor genes and bitter receptor genes. Mol Biol Evol 2006; 23(2): 292-300.
[http://dx.doi.org/10.1093/molbev/msj028] [PMID: 16207936]

[22] Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 2004; 432(7018): 695-716.
[http://dx.doi.org/10.1038/nature03154] [PMID: 15592404]

[23] Davis JK, Lowman JJ, Thomas PJ, *et al.* Evolution of a bitter taste receptor gene cluster in a New World sparrow. Genome Biol Evol 2010; 2: 358-70.
[http://dx.doi.org/10.1093/gbe/evq027] [PMID: 20624740]

[24] Medina-Tapia N, Ayala-Berdon J, Morales-Pérez L, Melo LM, Schondube JE. Do hummingbirds have a sweet-tooth? Gustatory sugar thresholds and sugar selection in the broad-billed hummingbird Cynanthus latirostris. Comp Biochem Physiol A Mol Integr Physiol 2012; 161(3): 307-14.
[http://dx.doi.org/10.1016/j.cbpa.2011.11.012] [PMID: 22155281]

[25] Zhao H, Zhang J. Mismatches between feeding ecology and taste receptor evolution: An inconvenient truth. Proc Natl Acad Sci USA 2012; 109(23): E1464.
[http://dx.doi.org/10.1073/pnas.1205205109] [PMID: 22562799]

[26] Matson KD, Millam JR, Klasing KC. Thresholds for sweet, salt, and sour taste stimuli in cockatiels (Nymphicus hollandicus). Zoo Biol 2001; 20(1): 1-13.
[http://dx.doi.org/10.1002/zoo.1001] [PMID: 11319776]

[27] Kudo K, Shiraishi J, Nishimura S, Bungo T, Tabata S. The number of taste buds is related to bitter taste sensitivity in layer and broiler chickens. Animal science journal = Nihon chikusan Gakkaiho 2010; 81: 240-4.

[28] Keller A, Zhuang H, Chi Q, Vosshall LB, Matsunami H. Genetic variation in a human odorant receptor alters odour perception. Nature 2007; 449(7161): 468-72.
[http://dx.doi.org/10.1038/nature06162] [PMID: 17873857]

[29] Davis RG. Olfactory psychophysical parameters in man, rat, dog, and pigeon. J Comp Physiol Psychol 1973; 85(2): 221-32.
[http://dx.doi.org/10.1037/h0035053] [PMID: 4756902]

[30] Clark L, Smeraski CA. Seasonal shifts in odor acuity by starlings. J Exp Zool 1990; 255(1): 22-9.
[http://dx.doi.org/10.1002/jez.1402550105] [PMID: 2391467]

[31] de Castro JM, Brewer EM. The amount eaten in meals by humans is a power function of the number of people present. Physiol Behav 1992; 51(1): 121-5.
[http://dx.doi.org/10.1016/0031-9384(92)90212-K] [PMID: 1741437]

[32] De Castro JM. Social facilitation of food intake in humans. Appetite 1995; 24(3): 260.
[http://dx.doi.org/10.1016/S0195-6663(95)99835-7] [PMID: 7574575]

[33] James WT. The development of social facilitation of eating in puppies. J Genet Psychol 1960; 96(1): 123-7.
[http://dx.doi.org/10.1080/00221325.1960.10534282] [PMID: 14406626]

[34] Sweeting MP, Houpt CE, Houpt KA. Social facilitation of feeding and time budgets in stabled ponies. J Anim Sci 1985; 60(2): 369-74.
[http://dx.doi.org/10.2527/jas1985.602369x] [PMID: 3988629]

[35] Dridi S, Raver N, Gussakovsky EE, *et al.* Biological activities of recombinant chicken leptin C4S analog compared with unmodified leptins. Am J Physiol Endocrinol Metab 2000; 279(1): E116-23.
[http://dx.doi.org/10.1152/ajpendo.2000.279.1.E116] [PMID: 10893330]

[36] Rolls BJ, Hetherington M, Burley VJ. The specificity of satiety: The influence of foods of different macronutrient content on the development of satiety. Physiol Behav 1988; 43(2): 145-53.
[http://dx.doi.org/10.1016/0031-9384(88)90230-2] [PMID: 3212049]

[37] Porrini M, Crovetti R, Riso P, Santangelo A, Testolin G. Effects of physical and chemical characteristics of food on specific and general satiety. Physiol Behav 1995; 57(3): 461-8.
[http://dx.doi.org/10.1016/0031-9384(94)00242-W] [PMID: 7753882]

[38] Rolls BJ, Laster LJ, Summerfelt A. Hunger and food intake following consumption of low-calorie foods. Appetite 1989; 13(2): 115-27.
[http://dx.doi.org/10.1016/0195-6663(89)90109-8] [PMID: 2802593]

[39] Thirumalaisamy G, Muralidharan J, Senthilkumar S, Sayee RH, Priyadharsini M. Cost-effective feeding of poultry. Int J Sci Environ Technol 2016; 5: 3997-4005.

[40] Jones GPD, Taylor RD. The incorporation of whole grain into pelleted broiler chicken diets: Production and physiological responses. Br Poult Sci 2001; 42(4): 477-83.
[http://dx.doi.org/10.1080/00071660120070587] [PMID: 11572623]

[41] Nir I, Shefet G, Aaroni Y. Effect of particle size on performance. 1. Corn. Poult Sci 1994; 73(1): 45-9.
[http://dx.doi.org/10.3382/ps.0730045] [PMID: 8165168]

[42] Zang JJ, Piao XS, Huang DS, Wang JJ, Ma X, Ma YX. Effects of feed particle size and feed form on growth perfromance, nutrient metabolizability and intestinal morphology in broiler chickens. Asian-Australas J Anim Sci 2009; 22(1): 107-12.
[http://dx.doi.org/10.5713/ajas.2009.80352]

[43] Dei HK, Bumbie GZ. Effect of wet feeding on growth performance of broiler chickens in a hot climate. Br Poult Sci 2011; 52(1): 82-5.
[http://dx.doi.org/10.1080/00071668.2010.540230] [PMID: 21337202]

[44] Forbes L, Graham J, Berglund C, Bell R. Dietary Change during Pregnancy and Women's Reasons for Change. Nutrients 2018; 10(8): 1032.

[http://dx.doi.org/10.3390/nu10081032] [PMID: 30096771]

[45] Olafsdottir AS, Skuladottir GV, Thorsdottir I, Hauksson A, Steingrimsdottir L. Maternal diet in early and late pregnancy in relation to weight gain. Int J Obes 2006; 30(3): 492-9.
[http://dx.doi.org/10.1038/sj.ijo.0803184] [PMID: 16331301]

[46] Spence C, Piqueras-Fiszman B. The perfect meal: the multisensory science of food and dining. 2014.
[http://dx.doi.org/10.1002/9781118491003]

[47] Piqueras-Fiszman B, Giboreau A, Spence C. Assessing the influence of the color of the plate on the perception of a complex food in a restaurant setting. Flavour (Lond) 2013; 2(1): 24.
[http://dx.doi.org/10.1186/2044-7248-2-24]

[48] Harrar V, Piqueras-Fiszman B, Spence C. There's more to taste in a coloured bowl. Perception 2011; 40(7): 880-2.
[http://dx.doi.org/10.1068/p7040] [PMID: 22128561]

[49] Piqueras-Fiszman B, Alcaide J, Roura E, Spence C. Is it the plate or is it the food? The influence of the color and shape of the plate on the perception of the food placed on it. Food Qual Prefer 2012; 24: 205-8.
[http://dx.doi.org/10.1016/j.foodqual.2011.08.011]

[50] Genschow O, Reutner L, Wänke M. The color red reduces snack food and soft drink intake. Appetite 2012; 58(2): 699-702.
[http://dx.doi.org/10.1016/j.appet.2011.12.023] [PMID: 22245725]

[51] Rosenthal N, Genhart M, Jacobsen FM, Skwerer RG, Wehr TA. Disturbances of appetite and weight regulation in seasonal affective disorder. Ann N Y Acad Sci 1987; 499(1): 216-30.
[http://dx.doi.org/10.1111/j.1749-6632.1987.tb36213.x] [PMID: 3300483]

[52] Rosenthal NE, Sack DA, Jacobsen FM, Skwerer RG, Wehr TA. Seasonal affective disorder & light: past, present and future. Clin Neuropharmacol 1986; 9 (Suppl. 4): 193-5.
[PMID: 3567939]

[53] Dark J, Rayha LL, Clark-Lane I, Kimler V. Melatonin and lighting condition: Absence of long-term effects on food intake and body weight regulation in the albino rat. Physiol Behav 1980; 25(6): 855-7.
[http://dx.doi.org/10.1016/0031-9384(80)90304-2] [PMID: 7220626]

[54] Ashwell M, Priest P, Bondoux M, Garrow JS. Resistance to slimming: adipose tissue cellularity studies. Proc Nutr Soc 1975; 34(2): 85A-6A.
[PMID: 1187646]

[55] Bellisle F, Dalix AM, Slama G. Non food-related environmental stimuli induce increased meal intake in healthy women: comparison of television viewing *versus* listening to a recorded story in laboratory settings. Appetite 2004; 43(2): 175-80.
[http://dx.doi.org/10.1016/j.appet.2004.04.004] [PMID: 15458803]

[56] Stroebele N, de Castro JM. Listening to music while eating is related to increases in people's food intake and meal duration. Appetite 2006; 47(3): 285-9.
[http://dx.doi.org/10.1016/j.appet.2006.04.001] [PMID: 16723172]

[57] Juslin PN, Sloboda JA. Music and emotion. The psychology of music (Elsevier Academic Press, San diego, CA, ed. 3rd Edition, ). 2013; p. 646.

[58] Bellisle F, Dalix AM. Cognitive restraint can be offset by distraction, leading to increased meal intake in women. Am J Clin Nutr 2001; 74(2): 197-200.
[http://dx.doi.org/10.1093/ajcn/74.2.197] [PMID: 11470720]

[59] North AC. The effect of background music on the taste of wine. Br J Psychol 2012; 103(3): 293-301.
[http://dx.doi.org/10.1111/j.2044-8295.2011.02072.x] [PMID: 22804697]

[60] Spence C. Auditory contributions to flavour perception and feeding behaviour. Physiol Behav 2012; 107(4): 505-15.

[http://dx.doi.org/10.1016/j.physbeh.2012.04.022] [PMID: 22579696]

[61] Spence C, Deroy O. On why music changes what (we think) we taste. Perception 2013; 4(2): 137-40. [http://dx.doi.org/10.1068/i0577ic] [PMID: 23755358]

[62] Ginane C, Baumont R, Favreau-Peigné A. Perception and hedonic value of basic tastes in domestic ruminants. Physiol Behav 2011; 104(5): 666-74. [http://dx.doi.org/10.1016/j.physbeh.2011.07.011] [PMID: 21798275]

[63] Curtis SE, Houpt KA. Animal ethology: its emergence in animal science. J Anim Sci 1983; 57: 234-47.

[64] Beauchemin KA. Invited review: Current perspectives on eating and rumination activity in dairy cows. J Dairy Sci 2018; 101(6): 4762-84. [http://dx.doi.org/10.3168/jds.2017-13706] [PMID: 29627250]

[65] Ferket PR, Gernat AG. Factors that affect feed intake of meat birds: A review. Int J Poult Sci 2006; 5(10): 905-11. [http://dx.doi.org/10.3923/ijps.2006.905.911]

[66] Quintana JA, Castaneda MP, Aguilera H, *et al.* Efecto de la altura de los comederos sobre el largo del tarso, pigmentación y parámetros productivos en pollo de engorda. Veterinaria (Mex) 1998; 29: 41-7.

[67] Roll VFB, Dai Prá MA, Xavier EG, *et al.* Efeito da altura do comedouro tubular sobre o desempenho e qualidade de carcaça em frangos de corte no período de 28 a 42 dias de idade. Cienc Anim Bras 2010; 11(4): 764-9. [http://dx.doi.org/10.5216/cab.v11i4.2989]

[68] Brobeck JR. Food intake as a mechanism of temperature regulation. Yale J Biol Med 1948; 20(6): 545-52. [PMID: 18872321]

[69] Collier RJ, Beede DK, Thatcher WW, Israel LA, Wilcox CJ. Influences of environment and its modification on dairy animal health and production. J Dairy Sci 1982; 65(11): 2213-27. [http://dx.doi.org/10.3168/jds.S0022-0302(82)82484-3] [PMID: 6759540]

[70] Johnson RE, Kark RM. Environment and Food Intake in Man. Science 1947; 105(2728): 378-9. [http://dx.doi.org/10.1126/science.105.2728.378] [PMID: 17841577]

[71] Cheung SS, McLellan TM, Tenaglia S. The thermophysiology of uncompensable heat stress. Physiological manipulations and individual characteristics. Sports Med 2000; 29(5): 329-59. [http://dx.doi.org/10.2165/00007256-200029050-00004] [PMID: 10840867]

[72] Westerterp-Plantenga MS, van Marken Lichtenbelt WD, Cilissen C, Top S. Energy metabolism in women during short exposure to the thermoneutral zone. Physiol Behav 2002; 75(1-2): 227-35. [http://dx.doi.org/10.1016/S0031-9384(01)00649-7] [PMID: 11890972]

[73] Bernhard MC, Li P, Allison DB, Gohlke JM. Warm Ambient Temperature Decreases Food Intake in a Simulated Office Setting: A Pilot Randomized Controlled Trial. Front Nutr 2015; 2: 20. [PMID: 26322311]

[74] Fletcher JM. Effects on growth and endocrine status of maintaining obese and lean zucker rats at 22°C and 30°C from weaning. Physiol Behav 1986; 37(4): 597-602. [http://dx.doi.org/10.1016/0031-9384(86)90292-1] [PMID: 3529146]

[75] Hamilton CL, Brobeck JR. Food intake and temperature regulation in rats with rostral hypothalamic lesions. Am J Physiol 1964; 207(2): 291-7. [http://dx.doi.org/10.1152/ajplegacy.1964.207.2.291] [PMID: 14205337]

[76] Jakubczak L. Food and water intakes of rats as a function of strain, age, temperature, and body weight. Physiol Behav 1976; 17(2): 251-8. [http://dx.doi.org/10.1016/0031-9384(76)90073-1] [PMID: 996163]

[77] Hamzaoui S, Salama AAK, Albanell E, Such X, Caja G. Physiological responses and lactational

performances of late-lactation dairy goats under heat stress conditions. J Dairy Sci 2013; 96(10): 6355-65.
[http://dx.doi.org/10.3168/jds.2013-6665] [PMID: 23958010]

[78] Rhoads ML, Rhoads RP, VanBaale MJ, *et al.* Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J Dairy Sci 2009; 92(5): 1986-97.
[http://dx.doi.org/10.3168/jds.2008-1641] [PMID: 19389956]

[79] Heath ME. Effect of rearing-temperature on the thermoregulatory behavior of pigs. Behav Neural Biol 1980; 28(2): 193-202.
[http://dx.doi.org/10.1016/S0163-1047(80)91551-4] [PMID: 7362583]

[80] Macari M, Zuim SMF, Secato ER, Guerreiro J. Effects of ambient temperature and thyroid hormones on food intake by pigs. Physiol Behav 1986; 36(6): 1035-9.
[http://dx.doi.org/10.1016/0031-9384(86)90476-2] [PMID: 3725906]

[81] Sohail MU, Hume ME, Byrd JA, *et al.* Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci 2012; 91(9): 2235-40.
[http://dx.doi.org/10.3382/ps.2012-02182] [PMID: 22912458]

[82] Deeb N, Shlosberg A, Cahaner A. Genotype-by-environment interaction with broiler genotypes differing in growth rate. 4. Association between responses to heat stress and to cold-induced ascites. Poult Sci 2002; 81(10): 1454-62.
[http://dx.doi.org/10.1093/ps/81.10.1454] [PMID: 12412909]

[83] Niu ZY, Liu FZ, Yan QL, Li WC. Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. Poult Sci 2009; 88(10): 2101-7.
[http://dx.doi.org/10.3382/ps.2009-00220] [PMID: 19762862]

[84] Imik H, Ozlu H, Gumus R, Atasever MA, Urcar S, Atasever M. Effects of ascorbic acid and $\alpha$ -lipoic acid on performance and meat quality of broilers subjected to heat stress. Br Poult Sci 2012; 53(6): 800-8.
[http://dx.doi.org/10.1080/00071668.2012.740615] [PMID: 23398425]

[85] Rajaei-Sharifabadi H, Greene E, Piekarski A, *et al.* Surface wetting strategy prevents acute heat exposure–induced alterations of hypothalamic stress– and metabolic-related genes in broiler chickens. J Anim Sci 2017; 95(3): 1132-43.
[http://dx.doi.org/10.2527/jas2016.1290] [PMID: 28380517]

[86] Tabler TW, Greene ES, Orlowski SK, Hiltz JZ, Anthony NB, Dridi S. Intestinal Barrier Integrity in Heat-Stressed Modern Broilers and Their Ancestor Wild Jungle Fowl. Front Vet Sci 2020; 7: 249.
[http://dx.doi.org/10.3389/fvets.2020.00249] [PMID: 32457922]

[87] Rajaei-Sharifabadi H, Ellestad L, Porter T, Donoghue A, Bottje WG, Dridi S. Noni (*Morinda citrifolia*) Modulates the Hypothalamic Expression of Stress- and Metabolic-Related Genes in Broilers Exposed to Acute Heat Stress. Front Genet 2017; 8: 192.
[http://dx.doi.org/10.3389/fgene.2017.00192] [PMID: 29259622]

[88] Morrison SF, Nakamura K. Central neural pathways for thermoregulation. Front Biosci 2011; 16(1): 74-104.
[http://dx.doi.org/10.2741/3677] [PMID: 21196160]

[89] Andersson B, Larsson B. Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. Acta Physiol Scand 1961; 52(1): 75-89.
[http://dx.doi.org/10.1111/j.1748-1716.1961.tb02203.x] [PMID: 13683301]

[90] Spector NH, Brobeck JR, Hamilton CL. Feeding and core temperature in albino rats: changes induced by preoptic heating and cooling. Science 1968; 161(3838): 286-8.
[http://dx.doi.org/10.1126/science.161.3838.286] [PMID: 4872449]

[91] Boulant JA, Gonzalez RR. The effect of skin temperature on the hypothalamic control of heat loss and heat production. Brain Res 1977; 120(2): 367-72.
[http://dx.doi.org/10.1016/0006-8993(77)90916-7] [PMID: 832130]

[92] Nagashima K, Nakai S, Tanaka M, Kanosue K. Neuronal circuitries involved in thermoregulation. Auton Neurosci 2000; 85(1-3): 18-25.
[http://dx.doi.org/10.1016/S1566-0702(00)00216-2] [PMID: 11189023]

[93] Nakamura K, Morrison SF. A thermosensory pathway that controls body temperature. Nat Neurosci 2008; 11(1): 62-71.
[http://dx.doi.org/10.1038/nn2027] [PMID: 18084288]

[94] Shlomo Y. Sturkie's Avian Physiology. Waltham, CA: Elsevier 2015; pp. 869-905.

[95] Bass D, Kleeman CR, Quinn M, Henschel A, Hegnauer AH. Mechanisms of acclimatization to heat in man. Medicine (Baltimore) 1955; 34(3): 323-80.
[http://dx.doi.org/10.1097/00005792-195509000-00002] [PMID: 13264957]

[96] Kozub FJ. Male-female differences in response to stomach loads of hypertonic NaCl in rats. Psychon Sci 1972; 28(3): 149-51.
[http://dx.doi.org/10.3758/BF03328690]

[97] Silanikove N. Effect of dehydration on feed intake and dry matter digestibility in desert (black bedouin) and non-desert (Swiss saanen) goats fed on lucerne hay. Comp Biochem Physiol A Comp Physiol 1985; 80(3): 449-52.
[http://dx.doi.org/10.1016/0300-9629(85)90066-0] [PMID: 2858325]

[98] Lautier JK, Dailey TV, Brown RD. Effect of water restriction on feed intake of white-tailed deer. J Wildl Manage 1988; 52(4): 602-6.
[http://dx.doi.org/10.2307/3800915]

[99] Viola TH, Ribeiro AML, Penz Júnior AM, Viola ES. Influence of water restriction on the performance and organ development of young broilers. Rev Bras Zootec 2009; 38(2): 323-7.
[http://dx.doi.org/10.1590/S1516-35982009000200015]

[100] Orlowski S, Flees J, Anthony N, Dridi S. Differential expression of water channel- and noncoding RNA biogenesis-related genes in three lines of chickens under a short-term water restriction. Poult Sci 2017; 96(12): 4172-81.
[http://dx.doi.org/10.3382/ps/pex263] [PMID: 29053842]

[101] Arad Z. Thermoregulation and acid-base status in the panting dehydrated fowl. J Appl Physiol 1983; 54(1): 234-43.
[http://dx.doi.org/10.1152/jappl.1983.54.1.234] [PMID: 6402471]

[102] Vij VA, Joshi AS. Effect of 'water induced thermogenesis' on body weight, body mass index and body composition of overweight subjects. J Clin Diagn Res 2013; 7(9): 1894-6.
[http://dx.doi.org/10.7860/JCDR/2013/5862.3344] [PMID: 24179891]

[103] Davy BM, Dennis EA, Dengo AL, Wilson KL, Davy KP. Water consumption reduces energy intake at a breakfast meal in obese older adults. J Am Diet Assoc 2008; 108(7): 1236-9.
[http://dx.doi.org/10.1016/j.jada.2008.04.013] [PMID: 18589036]

[104] Van Walleghen EL, Orr JS, Gentile CL, Davy BM. Pre-meal water consumption reduces meal energy intake in older but not younger subjects. Obesity (Silver Spring) 2007; 15(1): 93-9.
[http://dx.doi.org/10.1038/oby.2007.506] [PMID: 17228036]

[105] Boschmann M, Steiniger J, Hille U, *et al.* Water-Induced Thermogenesis. J Clin Endocrinol Metab 2003; 88(12): 6015-9.
[http://dx.doi.org/10.1210/jc.2003-030780] [PMID: 14671205]

[106] Boschmann M, Steiniger J, Franke G, Birkenfeld AL, Luft FC, Jordan J. Water drinking induces thermogenesis through osmosensitive mechanisms. J Clin Endocrinol Metab 2007; 92(8): 3334-7.

[http://dx.doi.org/10.1210/jc.2006-1438] [PMID: 17519319]

[107] Dubnov-Raz G, Constantini NW, Yariv H, Nice S, Shapira N. Influence of water drinking on resting energy expenditure in overweight children. Int J Obes 2011; 35(10): 1295-300.
[http://dx.doi.org/10.1038/ijo.2011.130] [PMID: 21750519]

[108] Macari M, Dauncey MJ, Ingram DL. Changes in food intake in response to alterations in the ambient temperature: Modifications by previous thermal and nutritional experience. Pflugers Arch 1983; 396(3): 231-7.
[http://dx.doi.org/10.1007/BF00587860] [PMID: 6844126]

[109] Langeveld M, Tan CY, Soeters MR, *et al.* Mild cold effects on hunger, food intake, satiety and skin temperature in humans. Endocr Connect 2016; 5(2): 65-73.
[http://dx.doi.org/10.1530/EC-16-0004] [PMID: 26864459]

[110] LeBlanc JA. in utritional Needs in Cold and High-Altitude Environments: Applications for Military Personnel in Field Operations B. M. C. Marriott, S.J., Ed. (National Academy Press, Washington) 1996; pp. 203-14.

[111] Bing C, Frankish HM, Pickavance L, *et al.* Hyperphagia in cold-exposed rats is accompanied by decreased plasma leptin but unchanged hypothalamic NPY. Am J Physiol 1998; 274(1): R62-8.
[PMID: 9458899]

[112] Holloszy J O, Smith E K. Longevity of cold-exposed rats: a reevaluation of the "rate-of-living theory" J Appl Physiol 1986; 61, 1656-1660 (1986). 61: 1656-60. (1986)

[113] Shefer VI, Talan MI. Change in heat loss as a part of adaptation to repeated cold exposures in adult and aged male C57bl/6J mice. Exp Gerontol 1997; 32(3): 325-32.
[http://dx.doi.org/10.1016/S0531-5565(96)00131-3] [PMID: 9193900]

[114] Ravussin Y, Xiao C, Gavrilova O, Reitman ML. Effect of intermittent cold exposure on brown fat activation, obesity, and energy homeostasis in mice. PLoS One 2014; 9(1): e85876.
[http://dx.doi.org/10.1371/journal.pone.0085876] [PMID: 24465761]

[115] Qureshi S, Khan HM, Mir MS, *et al.* Effect of Cold Stress and Various Suitable Remedies on Performance of Broiler Chicken. Journal of World's Poultry research 2018; 8: 66-73.

[116] Baarendse PJJ, Kemp B, Van Den Brand H. Early-age housing temperature affects subsequent broiler chicken performance. Br Poult Sci 2006; 47(2): 125-30.
[http://dx.doi.org/10.1080/00071660600610575] [PMID: 16641022]

[117] Shinder D, Ruzal M, Giloh M, Druyan S, Piestun Y, Yahav S. Improvement of cold resistance and performance of broilers by acute cold exposure during late embryogenesis. Poult Sci 2011; 90(3): 633-41.
[http://dx.doi.org/10.3382/ps.2010-01089] [PMID: 21325235]

[118] Nguyen P, Greene E, Ishola P, *et al.* Chronic Mild Cold Conditioning Modulates the Expression of Hypothalamic Neuropeptide and Intermediary Metabolic-Related Genes and Improves Growth Performances in Young Chicks. PLoS One 2015; 10(11): e0142319.
[http://dx.doi.org/10.1371/journal.pone.0142319] [PMID: 26569484]

[119] Drewnowski A, Shrager EE, Lipsky C, Stellar E, Greenwood MRC. Sugar and fat: Sensory and hedonic evaluation of liquid and solid foods. Physiol Behav 1989; 45(1): 177-83.
[http://dx.doi.org/10.1016/0031-9384(89)90182-0] [PMID: 2727131]

[120] Green BG. The effect of cooling on the vibrotactile sensitivity of the tongue. Percept Psychophys 1987; 42(5): 423-30.
[http://dx.doi.org/10.3758/BF03209749] [PMID: 3696937]

[121] Trant AS, Pangborn FM. Discrimination, intensity, and hedonic responses to color, aroma, viscosity, and sweetness of beverages. Lebensmittel Wissenschaff und Technologie 1983; 16: 147-52.

[122] Wurtman JJ. Carbohydrate craving, mood changes, and obesity. J Clin Psychiatry 1988; 49 (Suppl.):

37-9.
[PMID: 3045110]

[123] Bubenik GA, Pang SF, Cockshut JR, *et al*. Circadian variation of portal, arterial and venous blood levels of melatonin in pigs and its relationship to food intake and sleep. J Pineal Res 2000; 28(1): 9-15. [http://dx.doi.org/10.1034/j.1600-079x.2000.280102.x] [PMID: 10626596]

[124] Piccinetti CC, Migliarini B, Olivotto I, Simoniello MP, Giorgini E, Carnevali O. Melatonin and peripheral circuitries: insights on appetite and metabolism in Danio rerio. Zebrafish 2013; 10(3): 275-82.
[http://dx.doi.org/10.1089/zeb.2012.0844] [PMID: 23682835]

[125] Saarela S, Vuori M, Eloranta E, Vakkuri O. Melatonin, a candidate signalling molecule for energy sparing. Ornis Fenn 1999; 76: 231-5.

[126] Classen HL, Riddell C. Photoperiodic effects on performance and leg abnormalities in broiler chickens. Poult Sci 1989; 68(7): 873-9.
[http://dx.doi.org/10.3382/ps.0680873] [PMID: 2780476]

[127] Hester P, Sutton AL, Elkin RG, Klingensmith PM. The effect of lighting, dietary amino acids, and Utter on the incidence of leg abnormalities and performance of turkey toms. Poult Sci 1985; 64(11): 2062-75.
[http://dx.doi.org/10.3382/ps.0642062]

[128] Deep A, Schwean-Lardner K, Crowe TG, Fancher BI, Classen HL. Effect of light intensity on broiler production, processing characteristics, and welfare. Poult Sci 2010; 89(11): 2326-33.
[http://dx.doi.org/10.3382/ps.2010-00964] [PMID: 20952694]

[129] McCarthy DO, Kluger MJ, Vander AJ. The role of fever in appetite suppression after endotoxin administration. Am J Clin Nutr 1984; 40(2): 310-6.
[http://dx.doi.org/10.1093/ajcn/40.2.310] [PMID: 6465062]

[130] McCarthy DO, Kluger MJ, Vander AJ. Effect of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. Physiol Behav 1986; 36(4): 745-9.
[http://dx.doi.org/10.1016/0031-9384(86)90363-X] [PMID: 3520613]

# CHAPTER 4

# Regulation of Food Intake: Central Mechanisms

**Abstract:** Food intake is regulated by complex hypothalamic neuronal systems originally identified as hunger and satiety centers. The search for the underlying mechanisms led to the discovery of several central orexigenic and anorexigenic peptides. The present chapter summarizes the current knowledge about the role of classical hypothalamic neuropeptides such NPY/AgRP, POMC/CART, melanocortin, and orexin system, as well as several new central signals such as AMPK, ncRNA, and autophagy involved in the regulation of appetite and food intake.

**Keywords:** Hypothalamus, central signals, hunger center, satiety center, orexigenic neuropeptides, anorexigenic neuropeptides, ncRNA, autophagy.

## INTRODUCTION

Feed intake regulation is highly conserved across animals, and a series of highly integrated regulatory and neuronal mechanisms are involved. Brain lesioning and stimulation studies performed several decades ago involved the hypothalamus as a major site controlling feed intake and body weight. Early researchers found that lesions of the ventromedial hypothalamus (VMH) resulted in hyperphagia[1] and thereby defined it as the "satiety center" [1, 2], while lesions of the lateral hypothalamus area (LHA) resulted in aphagia[2] and thereby it was termed "hunger center" [3]. As in mammals, lesioning VMH of avian species increased feed intake, whereas lesioning LHA decreased feed intake [4]. As our knowledge of specific neuronal subpopulations involved in the regulation of feed intake has expanded, the term of brain "center" has been replaced by that of discrete neuronal pathways that generated responses to afferent inputs related to changing body fuel stores [5].

### 4.1. Classical Central Effector Pathways

The hypothalamus contains multiple neuronal systems essential in the regulation of feed intake. Stimulation of some of these systems results in a net increase in energy intake and storage and is thereby referred to as anabolic or orexigenic[3] systems. However, for others (catabolic or anorexigenic[4]), stimulation triggers a

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net decrease in feed intake and storage. In general, these classical hypothalamic effectors act similarly between mammals and avian species; however, there are notable exceptions (Table **4.1**).

### *4.1.1. NPY/AgRP and POMC/CART Neuropeptides*

Two separate populations of neuronal cell types are located in the mammalian arcuate nucleus (ARC, equivalent of infundibular nucleus in avian species); one synthesizes the powerfully orexigenic peptides (neuropeptide Y, NPY and agouti-related peptide, AgRP), while the other produces the anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) [5] (Fig. **4.1**). In both mammals and avian species, NPY is one of the most potent appetite stimulators. Central administration of NPY increases energy intake, decreases energy expenditure, and enhances adipogenesis and lipogenesis (*de novo* fatty acid synthesis, see chapter 9, section 9.4) [6 - 9]. Repeated central NPY administration induces obesity within a matter of days [10]. The effects of NPY on feed intake are mainly mediated *via* NPY receptor 2 (NPY Y2) and 5 (NPY Y5) [11], which belong to G-protein coupled receptors. The search for the underlying mechanism for the agouti obesity syndrome, in part led to the discovery of an endogenous melanocortin receptor (MCR) antagonist, AgRP. AgRP is co-expressed with NPY in the ARC, and when it is released from its neurons, it binds to MC3R and MC4R and, in turn, induces feed intake. Transgenic overexpression of AgRP produces an obesity syndrome [84]. Similarly, genetic deficiency of the MC4R in mice results in hyperphagia and obesity [85]. Unlike rodent models where prolonged effects of AgRP on feed intake have been reported [86], the effects of AgRP in sheep were not apparent after 24h. In pigs, neither AgRP nor SHU9119 (MC4-R antagonist) had any effects on feed intake thought to be due to a mutation in the MC4-R [87]. The expression and activity of NPY and AgRP are increased in conditions associated with weight loss, such as caloric restriction, lactation, and intense exercise [88 - 90] of particular interest among central catabolic systems are the melanocortins, neurotransmitters cleaved from the POMC precursor polypeptide. Mice lacking POMC are obese [91] and mutations that cause loss of POMC function in humans produce obesity and insulin resistance [92]. The alpha-melanocyte stimulating hormone (α-MSH), POMC-derived neuropeptide, is an endogenous MCR agonist that reduces feed intake when it acts on MC3R and MC4R [93]. Chronic infusion of α-MSH in the third cerebral ventricle of rats reduced feed intake and body weight [94]. Similarly, central administration of α-MSH suppresses feed intake in chickens [95]. In mammals, β-MSH binds MC4R with higher affinity than α-MSH, however, the opposite (α-MSH has a higher affinity to MC4R than does β-MSH) occurs in chicken [96, 97].

**Table 4.1. Comparison of neuropeptide effects on feeding behavior in mammalian and avian species.**

| (An)Orexigenic Peptides | Avian Species | Mammalian Species |
|---|---|---|
| ***Pancreatic Peptides*** | | |
| *NPY* | + [7, 12-16] | + [46-49] |
| *PP* | + [17] | - [50, 51] |
| *Peptide YY* | + [7] | - [52, 53] |
| ***Other Peptides*** | | |
| *AgRP* | + [18] | + [54] |
| *Galanin* | + [19] | + [55] |
| *Somatostatin* | + [20] | + [56] |
| *CGRP* | - [21] | - [57] |
| *CART* | - [22] | - [58] |
| *NPFF* | - [23] | - [59] |
| *NPK* | - [24] | - [60] |
| *NPS* | - | - [61] |
| *Mesotocin* | - [25] | - [62] |
| *Substance P* | - [26] | - [63] |
| *Vasotocin* | - [12] | - [64] |
| ***RFamide Peptides*** | | |
| *GnIH* | + [27] | + [65] |
| *26RFa* | + [28] | + [66] |
| *PrRP* | + [29] | - [67] |
| ***Melanocortins*** | | |
| *ACTH* | - [30] | - [68] |
| *A-MSH* | - [31] | - [69] |
| ***CRF Family*** | | |
| *CRH* | - [32] | + [70] |
| *Urotensin 1* | - [33] | + [71] |
| *Urocortin* | - [34] | + [72] |
| *Stresscopin* | - [35] | + [73] |
| ***Glucagon Family*** | | |
| *GLP-1/2* | - [36] | - [74, 75] |
| *Oxyntomodulin* | - [37] | - [76] |
| *GHRH* | - [38] | + [77] |
| ***Brain-gut Peptide*** | | |

*(Table 1) cont.....*

| | | |
|---|---|---|
| *CCK* | - [39] | - [78] |
| *Gastrin* | - [40] | - [79] |
| *GRP* | - [41] | - [80] |
| *Neuromedin B/C/S/U* | - [42] | - [81] |
| ***Opioid Family*** | | |
| *B-endorphin* | + [43] | + [82] |
| *Endomorphin-2* | + [44] | + [44] |
| *Nociceptin* | + [45] | + [83] |

ACTH, adrenocorticotropic hormone; AgRP, agouti related peptide; α-MSH, alpha-melanocyte stimulating hormone; CART, cocaine-amphetamine-regulated transcript; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; CRH, corticotropin-releasing hormone; GHRH, growth hormone releasing hormone; GLP1/2, glucagon-like peptide1/2; GnIH, gonadotropin-inhibiting hormone; GRP, gastrin-releasing peptide; NPFF, neuropeptide FF; NPS, neuropeptide S; NPY, neuropeptide Y; PP, pancreatic peptide; PrRP, prolactin-releasing peptide; +, stimulates; -, inhibits.

Another classical anorexigenic neuropeptide is CART which is also found in POMC neurons in the ARC. However, in the PVN, CART mRNA was found to be localized with vasopressin and corticotropin-releasing factor (CRF)-containing neurons in rats [98]. When injected intracerebroventricularly[5] into rats and chickens, recombinant CART peptide inhibits both normal and starvation-induced feeding [22, 58].

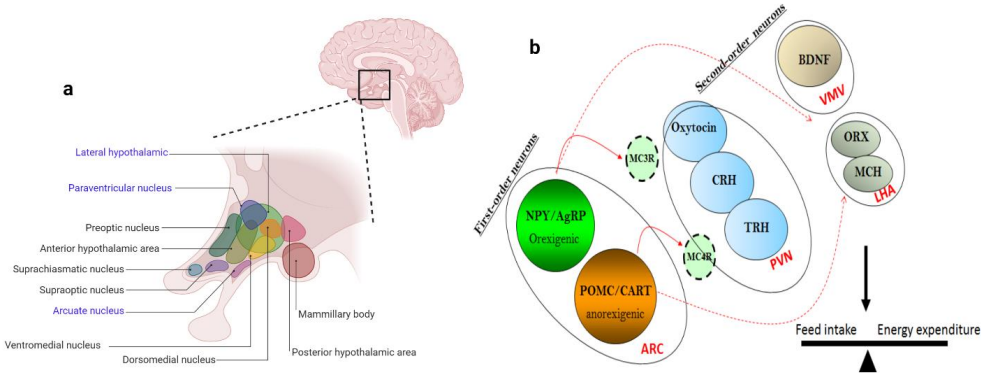


**Fig. (4.1). Myth of hypothalamic hunger and satiety centers and feeding-related (an)orexigenic neuropeptides**. Sagittal diagram of the brain outlines the major hypothalamic centers involved in the control of energy balance (a, b). Populations of first-order orexigenic NPY/AgRP and anorexigenic POMC/CART neurons are located in the ARC and they project to the PVN, LHA, and VMH, which are the location of second-order hypothalamic neuropeptide neurons involved in the regulation of feed intake and energy homeostasis. AgRP, agouti-related peptide; ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; CART, cocaine and amphetamine regulated transcript; CRH, corticotropin-releasing hormone; DMH, dorsomedial hypothalamus; LHA, lateral hypothalamus; MCH, melanin-concentrating hormone; MC3/4R, melanocortin receptor 3 and 4; NPY, neuropeptide Y; ORX, orexin; POMC, proopiomelanocortin; PVN, paraventricular nucleus; TRH, thyrotropin-releasing hormone; VMH, ventromedial hypothalamus.

### *4.1.2. Melanocortin System*

Although there are 5 MCRs, MC3R and MC4R are the key players in the regulation of energy homeostasis. MC3R and MC4R mRNA are expressed in several hypothalamic areas involved in regulating feeding behavior such as ARC, PVN, and LHA as well as in numerous extra-hypothalamic sites [99]. As mentioned above, MC4R blockade causes an obesity phenotype and MC4R deficient mice and humans are obese. Recent studies suggest that the melanocortin system may contribute not only to the homeostatic regulation of feed intake but also to its hedonic aspects *via* the MC4R inputs to the nucleus accumbens [100]. Interestingly, MC3Rs are also expressed in limbic regions involved in controlling ingestive behaviors and autonomic function. It seems that increased adiposity and accelerated diet-induced obesity in MC3R knockout mice to be hyperphagia-independent. Several studies showed that MC3R-/- mice displayed reduced physical activity and locomotor behavior, suggesting a key role of MC3R in the regulation of energy expenditure [101]. This hypothesis was supported by the observation that fatty acid oxidation and citrate synthase activity are reduced in skeletal muscle of female MC3R deficient mice, indicating reduced mitochondrial activity [102]. However, the mechanisms underlying the differences in mitochondrial activity have not been completely defined.

### *4.1.3. Melanin-concentrating Hormone (MCH)*

The mammalian form of MCH, is a 19-amino acid cyclic peptide encoded within a 165-amino acid prepro-MCH. The MCH preprohormone can generate, through alternative processing, two additional putative peptides, designated neuropeptide E-I (NEI) and neuropeptide G-E (NGE) with unknown functions [103]. Several *in vivo* studies have shown that MCH plays a role in a variety of physiological processes, including energy homeostasis, sleep, arousal, and emotionality. Neurobiological, genetics and pharmacological studies demonstrate that MCH is an orexigenic peptide which induces obesity [104]. Investigation on the effector mechanisms by which MCH is orexigenic has largely focused on the MCHR1 receptor in the nucleus accumbens shell, which has been thought to be involved in motivational aspects of eating. MCRH2 is absent in rodents but is present in higher species such as primates [105]. Although they are hyperphagic on high-fat diet (HFD), MCHR1 knockout mice are resistant to obesity, which is due to hyperactivity and higher metabolic rate [106].

### *4.1.4. Orexins/hypocretins*

The neuropeptides orexin A and orexin B (also known as hypocretin 1 and hypocretin 2, respectively) are produced in cell bodies of the LHA, but have extensive projections to many brain regions, including the ARC [107]. They bind

to two G-protein-coupled receptors, orexin receptors 1 and 2 (ORXR1 and ORXR2), which are encoded by two separate genes [108]. Orexins are appetite-stimulating neuropeptides in mammals, but they also have several other physiological functions such as sleep-wakefulness, stress, anxiety, and energy metabolism.

Central administration of orexin stimulates feed intake in mammals [108]. However, an important difference in the effects on feeding between orexin and other feeding-related hypothalamic neuropeptides such as NPY and MCH, is that orexin increases both energy intake and expenditure, whereas the others generally decrease energy expenditure [109]. It must be recognized that multiple peptide systems interact to control the feeding response, and the hierarchy within those systems is not completely defined. For instance, studies suggested that NPY neurons are located downstream to orexin; however other evidence does exist for the opposite diagram where orexin neurons were found to be located downstream to NPY [110]. Similarly, ICV injection of orexin increases feeds intake in sheep at 2 and 4h post-administration [111]. Intriguingly, the central administration of orexin did not affect feed intake in neonatal chicks [112], suggesting that orexin may play other physiological roles in avian species. Recently, our laboratory found that the orexin system is expressed in muscle and regulates muscle energy metabolism in avian species [113].

### *4.1.5. Galanin*

Galanin is a 29-amino acid C-terminally amidated, found in both the CNS (PVN and ARC nuclei) and intestine, is conserved across species. In the brain, galanin is co-expressed with NPY. Central administration of galanin elicits a potent feeding response in mammals [114]. Furthermore, administration of galanin in the PVN area causes a macronutrient selection with a preferential increase in the consumption of the fat diet compared to carbohydrates and proteins [115]. There are two characterized galanin subtype receptors: GalR1 and GalR2, which are majorly distributed in the hypothalamus, brainstem, hippocampus, spinal cord, and many other tissues [116]. It has also been reported that mammalian galanin participate in modulating learning, memory, inflammation, sexual behavior, insulin, and pituitary hormone release [117, 118]. In birds, the effects of galanin on feeding are controversial. In fact, Tachibana and coworkers have shown that ICV administration of galanin stimulates feed intake but not water consumption in both broiler (meat-type) and layer (egg-type) chickens [119]. In contrast, Ando and colleagues reported that ICV injection of galanin does not increase feed intake in neonatal chicks [120]. The effects of galanin on feeding seem to be mediated *via* μ-opioid receptors and α2 adrenoceptors.

### *4.1.6. Galanin-like Peptide (GALP)*

Galanin-like peptide is a 60-amino acid peptide, with residues 9-21 being identical to the biologically active N-terminal (1-13) portion of galanin [121]. By using double-label *in situ* hybridization, Cunningham *et al.* [122] found that GALP-containing neurons in primates express the NPY receptor 1, indicating that NPY may regulate GALP neurons in the ARC. Central infusion of GALP into the rat lateral ventricle increases feed intake in the first 1-2h, and this effect was 10 times higher than that of galanin [123, 124].

It has been postulated that the effect of GALP on rat hypothalamus could be mediated by an increase in NPY release and a decrease in CART release [125]. *In vivo* chronic injection of GALP reduced POMC expression in the ARC of ob/ob mouse [126]. Kageyama *et al.* [127] showed that blocking orexin action with an anti-orexin antibody inhibited GALP-induced hyperphagia, indicating that orexin mediated the effects of GALP. The receptor responsible for the orexigenic effect of GALP in rodents is likely to be GALR1, and there is no evidence to support a role for GALR2 or GALR3.

### *4.1.7. Cerebellin 1*

Cerebellin 1 (Cbln1) is highly expressed in the mammalian hypothalamus. Central administration of Cbln1 stimulated feed intake and the release of NPY, suggesting that the orexigenic effects of Cbln1 are probably mediated through hypothalamic NPY [128]. Reiner *et al.* [129] previously identified the chicken homolog of Cbln2 and found that it is frequently expressed in primary sensory neurons and second-order sensory regions. However, the effect of Cbln1 in feeding behavior in chickens is unknown yet.

### *4.1.8. Glucagon-like Peptide*

When it is released from the nucleus of the solitary tract and from neuronal projections to the PVN, glucagon-like peptide-1 (GLP-1) activates its receptor and promotes satiety and anorexia. Furthermore, activated GLP-1 neurons also projected to the ARC and modulated vagal motor outflow to the pancreas, leading to an increase in insulin secretion and reduction of glucagon levels and thereby lowering blood glucose levels. Peripheral (intravenous) administration of GLP-1 in normal and obese humans inhibited feed intake in a dose-dependent manner and reduced gastric emptying [130]. GLP-2 is co-localized with GLP-1, and it is a potent inhibitor of feed intake when centrally injected [131]. GLP-2 receptor

knockout in POMC neurons increased feed intake, which is mainly due to increased gastric motility and increased meal frequency, indicating that central GLP-2 is a key satiety signal. Guan and coworkers demonstrated that this effect was mediated by MC4R [132]. The anorexigenic effects of GLP-1 and GLP-2 have also been observed in chickens [133, 134].

### *4.1.9. Corticotropin-releasing Factor (CRF)*

Corticotropin-releasing factor or corticotropin-releasing hormone (CRF or CRH, respectively) is a 41-amino acid mammalian neuropeptide that is best known as the key physiological regulator of pituitary adrenocorticotropic hormone (ACTH) secretion. Central administration of CRF inhibited feed intake and body weight in rodents [71].

Similarly, CRH acted within the brain to inhibit feed intake in both broilers and layers [32], and this effect seemed to be mediated *via* CRH receptor. Moreover, blocking nitric oxide has been shown to attenuate the effect of CRH on feeding in chickens [135].

### *4.1.10. Neurotensin*

The 13-amino acid peptide, neurotensin, is mainly produced in the ARC, PVN, and DMH and is anorexigenic when injected centrally in mammals [136]. In the chicken, neurotensin is produced in both the brain and intestine. Masuda *et al.* [137] found high expression of neurotensin in the hypothalamic infundibulum and have reported that central administration of neurotensin had no effect on feed intake in chickens, suggesting that the effect of neurotensin on feeding behavior is species-specific.

### *4.1.11. Nesfatin 1*

Although three nesfatin peptides; nesfatin 1, 2, and 3 have been identified, only nesfatin 1 is known to be biologically active [138]. Administration of the bioactive core of nesfatin 1 inhibited feed intake and reduced body weight in rodents [138]. The effects of nesfatin 1 seem to be mediated by direct inhibition of ARC neurons containing NPY. Nesfatin 1 is also likely to act on oxytocin, CRF2, and MCR3/4 to reduce feed intake [139]. The contention that nesfatin 1 is an appetite suppressive molecule has been further confirmed in avian species, where peripheral and central administration of nesfatin 1 has been shown to decrease feed intake in quails [140].

### *4.1.12. FMR Famides*

Several RFamide peptides have been characterized and identified as key players in the regulation of feed intake. For instance, prolactin-releasing peptide reduced feed intake and mediated satiety signaling in rodents [141], however, it stimulated feed intake when centrally injected in chickens [29]. Wang and co-workers have shown that this orexigenic effect of prolactin-releasing peptide is associated with hypothalamic NPY [142]. Similarly, central administration of gonadotropin-inhibiting hormone-induced feeding behavior in chickens and this effect is likely mediated *via* μ-opioid receptor [27].

Central administration of 26RFa, another RFamide, stimulated appetite in broilers but not in layers, indicating strain-specific effects [28]. Likewise, several members of the neuropeptide FF subfamily of the RFamide have also been shown to decrease feed intake in chickens [23].

Many other (an)orexigenic neuropeptides have been identified, and their effect on feed intake in both mammalian and avian (non-mammalian) species are summarized in **Table 4.1.**

## 4.2. New Central Molecular Pathways

### *4.2.1. Neurosecretory Protein GL and GM (NPGL and NPGM)*

With the advances in molecular approaches and sequencing technologies, new hypothalamic neuropeptides were identified, including NPGL and NPGM. These neuropeptides have been characterized in humans, rats, chickens, and several other species, and they are highly conserved (Fig. **4.2**) [143]. Each of these precursor proteins contains a signal peptide at the N-terminus, a glycine amidation signal, and a dibasic amino acid cleavage site [143]. It has been shown that the mediobasal hypothalamic expression of NPGL increased with the age of chickens [144] and that chronic ICV[6] administration of NPGL induced feed intake and water consumption [145].

Although NPGL regulates feeding behavior in chickens, its effect seems to be independent of classical neuropeptides. Indeed, Shikano and coworkers reported that NPGL administration did not affect the expression of NPY, AgRP, POMC, GLP-1, or CCK [145]. In mice, ICV administration of NPGL increased feed intake and this effect was hypothesized to be mediated at least *via* inhibition of POMC [147]. Similarly, adenovirus-mediated overexpression of NPGL increased feed intake and body mass in rats [148].

Interestingly, NPGL and its paralog NPGM are co-localized in the chicken IN[7] (the equivalent of mammalian ARC), however and in contrast to NPGL, ICV administration of NPGM decreased feed intake in chicken [149].

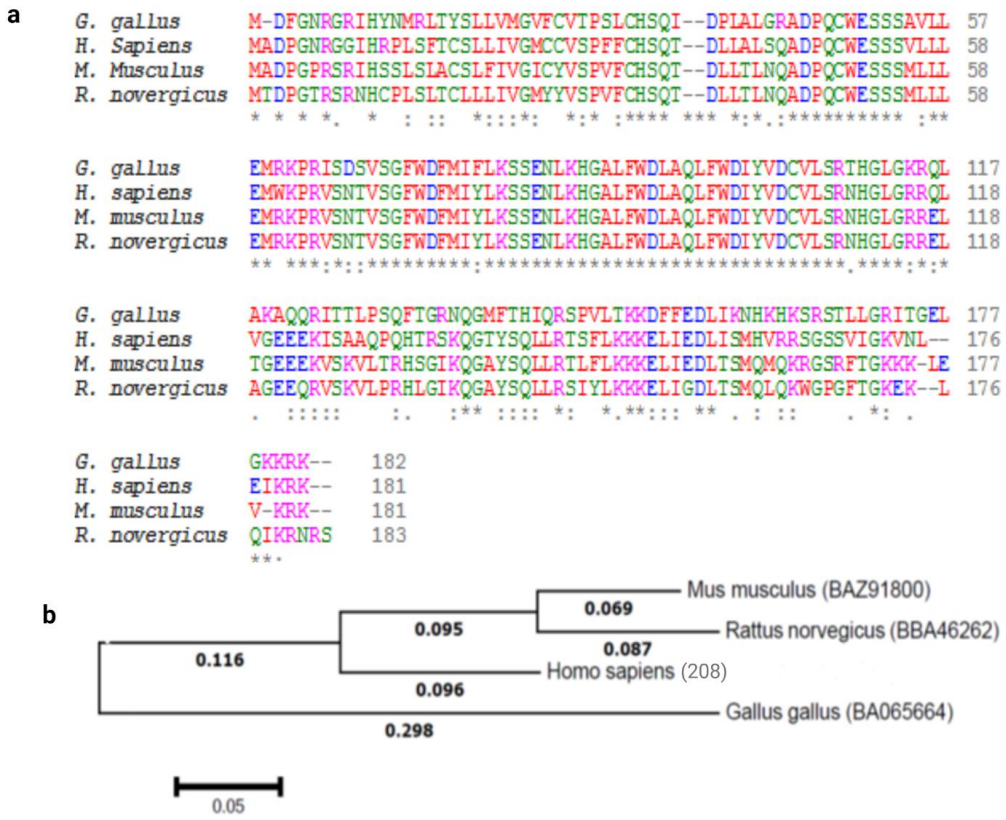


**Fig. (4.2). Alignment and phylogeny tree of NPGL.** Amino acid sequence alignment of NPGL from chicken (gallus gallus, BA065664), human (homo sapiens, obtained from (208)), mouse (*mus musculus*, BAZ91800), and rats (*rattus norvegicus*, BBA46262) (a). The evolutionary distances and the phylogenetic tree of NPGL (b) were inferred using the Neighbor-Joining method in MEGA6 [146]. Identical (*) and similar (:) amino acid residues are indicated.

### *4.2.2. AMP-activated Protein Kinase (AMPK) Pathway*

AMPK is a serine/threonine kinase that is evolutionarily conserved from yeast to mammals. It is a heterotrimeric complex consisting of a catalytic α subunit and regulatory β/γ subunits, each of which is encoded by two or three distinct genes (α1, α2, β1, β2, γ1, γ2, and γ3). AMPK is a master cellular fuel gauge and energy sensor [150]. In fact, as described in previous chapters, every living cell contains a "rechargeable battery" formed by interconverted ATP and ADP according to the

following reaction: ATP ↔ ADP + phosphate, and every cellular function requires energy; thus ATP generation needs to remain in balance with ATP consumption. However, under stress conditions that deplete cellular energy (inhibition of ATP production or increase of ATP consumption), the AMP/ATP and ADP/ATP ratios increase, which in turn activate AMPK by binding to the regulatory nucleotide-binding domains of the AMPKγ subunit (Fig. **4.3**). Activation of AMPK represses ATP-consuming anabolic pathways and induces ATP-producing catabolic pathways at cellular levels. AMPK also regulates energy balance at the whole body level mainly *via* effects on the hypothalamus and feed intake.

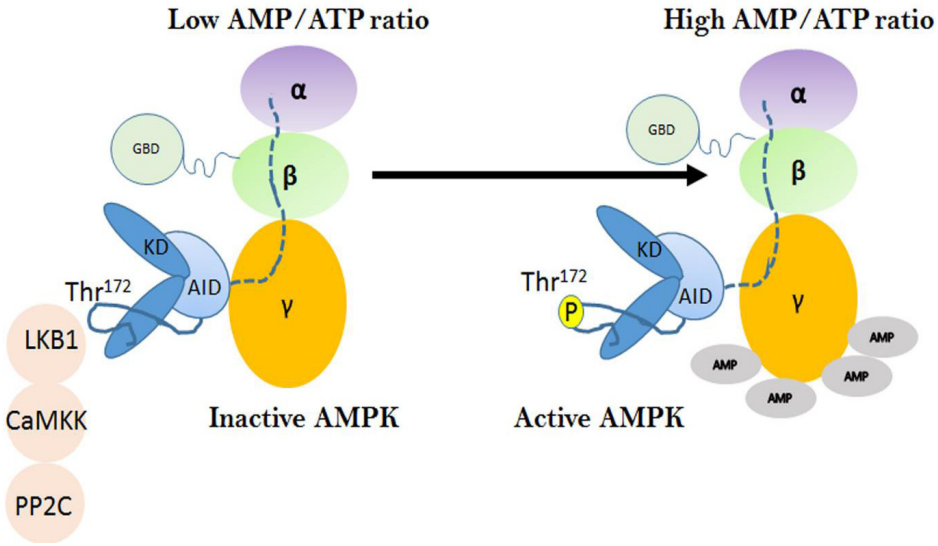


**Fig. (4.3). Illustration of AMPK activation.** AMPK is a heterotrimeric complex of α, β, and γ subunits. When cellular ATP levels are high, AMP/ATP ratio decreases, and little AMP is bound to γ subunit, and AMPK is in its inactive form. When the AMP/ATP ratio increases with energetic stress, more AMP is bound to the γ subunit and the AID subunit releases from the KD and AMPK is phosphorylated at $Thr^{172}$ site and is activated. AID, autoinhibitory domain; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; GBD, glycogen binding domain; KD, bilobar kinase domain.

Central injection of pharmacological activator of AMPK or DNA encoding activated mutant stimulated feed intake [151, 152]. Changes in hypothalamic AMPK activity resulted in alteration of hypothalamic feeding-related neuropeptides. Indeed, overexpression of dominant-negative AMPK in medial basal hypothalamus suppressed NPY and AgRP expression in the ARC, whereas overexpression of constitutively active AMPK stimulated NPY/AgRP in the ARC and MCH in the LHA [152]. Together, these studies indicated that the effect of AMPK on feeding behavior is likely to be mediated *via* the hypothalamic neuropeptides.

To identify specific hypothalamic neurons in which AMPK mediated its effect on feeding behavior, Claret and coworkers generated mice lacking the α2 catalytic subunit of AMPK specifically in POMC- or AgRP-expressing neurons and found a reduced body weight in AgRP-AMPKα2-KO[8], but increased body weight and adiposity in POMC-AMPKα2-KO mice [153].

These divergent effects suggest that AMPK plays specific roles in specific neuronal populations.

In chickens, AMPK subunits were characterized in 2006 by McMurtry's group [154]. Nutritional states (fasting and refeeding) have been reported to modulate hypothalamic AMPK expression [155]. Xu *et al.* [156] have shown that administration of AICAR (AMPK inducer) and compound C (AMPK inhibitor) affected feed intake independently of AMPK activation. Dridi's group has shown that ICV administration of leptin activated hypothalamic AMPKα1/2 at Thr172 site and reduced feed intake in chickens [157]. Together, these data suggested that AMPK is responsive to the nutritional status and that AMPK regulated feed intake in chickens. However, further in-depth studies are warranted to determine the up- and down-stream mediators of avian AMPK. The changes in neuropeptides following AMPK activation are similar to those observed with ICV administration of fatty acids or manipulation of hypothalamic fatty acids [158].

### *4.2.3. Hypothalamic Fatty Acids*

The role of central fatty acid metabolism in the regulation of energy homeostasis has attracted the attention of many researchers and has become a hot spot research area. Systemic administration of fatty acids gains access to various brain regions [159]. Both passive diffusion and protein carrier (cluster of differentiation 36, CD36 and fatty acid transporter 1, FATP1)-mediated models for the translocation of plasma fatty acids across the blood-brain barrier (BBB) have been proposed. Once they gain access to the cytosolic compartment, these fatty acids generate fatty acyl-CoA molecules *via* activation of acyl-CoA synthetase enzyme. These generated fatty acyl-CoA molecules can be uptaken by the mitochondria for β-oxidation (the rate is very low in the brain), or converted into phospholipids, triacylglycerol, and fatty acyl carnitine.

Le Foll *et al.* [160] have shown that the hypothalamus contains "metabolic sensing neurons" which monitor substrate levels such as fatty acids. These neurons alter their membrane potential and activity in response to these substrate levels and regulate feed intake. Several studies have shown that fatty acid-sensing neurons are localized in the VMN and ARC [161]. In 2009, Jo and co-workers [162] showed that 40% of ARC POMC neurons sense long-chain fatty acids. Further, ICV administration of the long-chain fatty acid oleic acid (OA) inhibited

feed intake in rodents *via* an increase in hypothalamic POMC mRNA expression and blocking MC4R completely reversed the anorectic effect of OA [163]. Obici *et al.* [164], however, reported that central administration of OA in rats inhibited food intake independently of leptin but was accompanied by a decrease in the hypothalamic expression of NPY. These data indicated that fatty acids regulated feed intake *via* modulation of hypothalamic (an)orexigenic neuropeptides (Fig. **4.4**). It has been also suggested that fatty acids can signal nutrient availability to the CNS and that their esterification to LCFA-CoAs is a key step for their central anorexigenic action [165], because inhibition of esterification by triascin C blocked the anorexic effects of ICV LCFA.

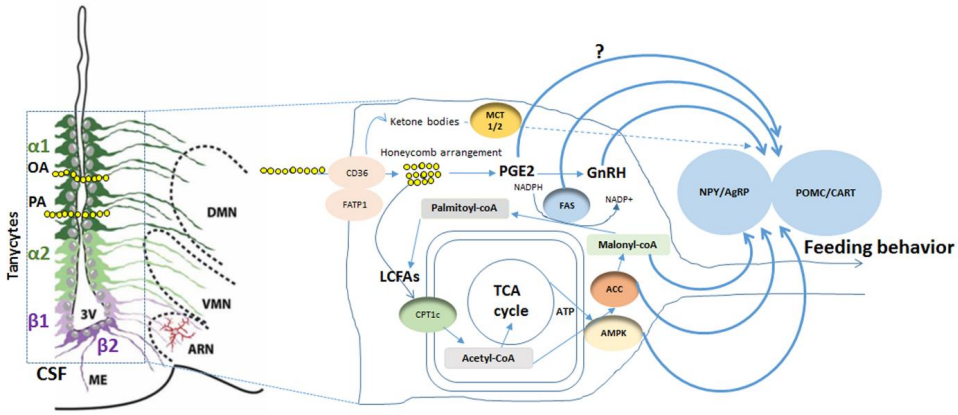


**Fig. (4.4). Model and potential mechanisms of central fatty acid sensing and detection in feeding behavior**. Fatty acids cross the BBB and are taken up *via* both passive diffusion and protein carriers (CD36, FATP1). During fasting or low blood glucose levels, FFAs are elevated, metabolized by astrocytes, and enter the mitochondria *via* CPT1c for β-oxidation and ATP production. During HFD, hypothalamic astrocytes produce ketone bodies from FAs, which in turn are uptaken into neurons by MCT2 and are then metabolized in the mitochondria to produce ATP. Another type of glial cell, tenocytes, has been found to play a crucial role in central fatty acid sensing, storage, and metabolization with the production of PGE2 and GnRH; both were reported to regulate feeding behavior. FAs in the brain can also originate from *de novo* synthesis, with the first step being the production of malonyl-CoA from acetyl-CoA (comes from citrate produced in the mitochondria), a reaction catalyzed by ACC. Malonyl-CoA is then catalyzed by FAS to produce palmitoy-CoA, LCFAs, and LCFA-CoA, all of which are the regulator of feed intake. ACC, acetyl-CoA carboxylase; AgRP, agouti-related protein; AMPK, AMP-activated protein kinase; ARN, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; CD36, cluster of differentiation 36; CPT1c, carnitine palmitoyltransferase 1c; CSF, cerebrospinal fluid; DMN, dorsomedial hypothalamic nucleus; FAS, fatty acid synthase; FATP1, fatty acid transport protein 1; GnRH, gonadotropin-releasing hormone; MCT1/2, monocarboxylate transporter; OA, oleic acid: PA, palmitic acid; PGE2, prostaglandin 2; POMC, proopiomelanocortin; VMN, ventromedial nucleus

The fatty acid translocase CD36 has been found to be a major regulator of neuronal fatty acid sensing and food intake [166]. Depletion of CD36 in the ventromedial hypothalamus (VMH) of diet-induced obese (DIO) rats fed 45% high-fat diet increased their food intake and body weight gain compared to DIO

controls [167]. More recently, several molecular pathways involved in the fatty acid synthesis, lipolysis, or oxidation have been reported to regulate feed intake.

### *4.2.3.1. Central De-novo Fatty acid Synthesis*

In addition to peripheral (derived from the diet) source, central LCFAs can result from local hypothalamic *de-novo* synthesis. The synthesis of fatty acid is a cytosolic process, which involves two key enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN). ACC carboxylates acetyl-CoA (which comes from citrate produced in the mitochondria by the citric acid cycle) to form malonyl-CoA. The malonyl-CoA is further converted, by FASN, to form LCFAs. Conversely, malonyl-CoA can be decarboxylated into acetyl-CoA and $CO_2$ by malonyl-CoA decarboxylase (MCD) and results in a decrease in the *de-novo* fatty acid synthesis. Thus, the generation of a new LCFA molecule in the hypothalamic ARC depends heavily on the metabolism of glucose to produce the necessary substrates (acetyl-CoA, malonyl-CoA, and NADPH, which is a cofactor of FASN). It is noteworthy that when phosphorylated, ACC is inhibited whereas MCD is activated, two events that lower malonyl-CoA levels. Previous studies, including our own ones, showed that the abovementioned enzymes are expressed in the hypothalamus [168]. Intraperitoneal or ICV administration of FAS inhibitors (C75 or cerulenin) increased hypothalamic malonyl-CoA concentration, reduced food intake and body weight, and increased energy expenditure [169, 170]. These effects were also accompanied by increased activation of neurons in the hypothalamic ARC, decreased expression of NPY and AgRP, and increased expression of POMC and αMSH [171, 172]. The two isoforms, ACC1 and ACC2, outer mitochondrial membrane proteins, were also found in the hypothalamus. Interestingly, ICV administration of 5-(tetradecyloxy)-2-furoic acid (TOFA), an inhibitor of ACC, prior injection of C75, blocked the anorectic effect of C75 [169]. Although both TOFA and C75 inhibit fatty acid synthesis, TOFA antagonized the anorectic effect of C75. This led to the hypothesis that hypothalamic malonyl-CoA plays a key role in the control of feeding behavior [173], because C75 increased and TOFA reduced malonyl-CoA levels. Further nutritional (fasting and refeeding) studies confirmed this hypothesis. Following fasting (negative energy balance status), malonyl-CoA levels dropped and stimulated food intake however refeeding would limit the rebound feeding response *via* an increase of malonyl-CoA levels. Further hormonal (leptin, ghrelin) studies showed the key role of AMPK-ACC-malonyl-CoA axis in the regulation of food intake [152, 174, 175]. Leptin administration inhibited hypothalamic AMPK activity, activated ACC, increased malonyl-CoA levels, and reduced food intake. Blockade of ACC by TOFA, or overexpression of MCD prevented leptin's anorectic action [152, 176, 177]. Ghrelin stimulated appetite

and food intake *via* AMPK activation, ACC inhibition, and malonyl-CoA decrease in the hypothalamus [175].

#### ***4.2.3.2. Central Fatty Acid Oxidation and Carnitine Palmitoyl-transferase (CPT)***

The two isoforms of CPT1, CPT1a in the liver and CPT1b in the muscle, catalyze the first step of the translocation of LCFA-CoAs into mitochondria for β-oxidation. Usually, the brain relies on glucose as a primary fuel source when carbohydrate is available or ketones during starvation and high-fat feeding. Although neural tissues do not normally use fatty acids as a major physiological fuel, a recent study has characterized a novel CPT1c isoform predominantly expressed in the brain [178]. Initial studies showed that CPT1c did not possess acyltranferase activity and did not support fatty acid translocation and oxidation in mitochondria of hypothalamic explants, nor in cell culture [178], however, it binds to malonyl-CoA. In a later study, Sierra *et al.* [179] showed that brain CPT1c had a very weak acyltransferase activity and preferentially used palmitoyl-CoA as a substrate. Further studies have shown that central CPT1c plays a key role in the regulation of food intake. Indeed, CPT1c knockout mice fed with a regular chow diet have reduced food intake [180], while overexpression of CPT1c in the hypothalamic ARC increased food intake [181]. In addition, ARC overexpression of CPT1c blocked leptin or cerulenin-induced anorectic effects. Carrasco and colleagues have shown that brain CPT1c regulated ceramide *de novo* biosynthesis [182], and both CPT1c and ceramide are downstream mediators in malonyl-CoA action on food intake.

#### ***4.2.3.3. Central Lipolysis and Lipoprotein Lipase (LPL)***

Lipoproteins are produced within the CNS and contribute to the brain's lipid sensing [183]. They are triglyceride-enriched particles, and they are hydrolyzed by lipases. Lipoprotein lipase is expressed in the brain [184], and depletion of LPL in the VMH induced body weight gain in rodents [185]. Depletion of LPL in the dorsal hippocampus, however, increased body weight gain without affecting food intake in rodents [186]. These studies indicated that the increase in body weight gain was associated with a decrease in energy expenditure and locomotor activity.

### ***4.2.4. Hypothalamic Glucose***

Maintaining a healthy body weight requires balancing food intake, energy and nutrient partitioning and deposition, and energy expenditure. This energy homeostasis necessitates an ability of the brain to detect the status of energy stores and match energy intake with energy expenditure. In 1953, Jean Mayer was the

first to propose the "glucostatic hypothesis" and postulated that reduced glucose utilization in critical brain area leads to perception and expression of hunger, however, increased glucose utilization in the same area induces satiety and cessation of eating [187]. Subsequent studies have shown that fall in blood glucose was correlated with meal initiation in both humans and rodents [188 - 190] and argued that glucose accounts for the short-term control of feed intake. However, lipostatic theory (described above) account for the long-term regulation of body weight and energy balance [191]. Bray [192] revisited Mayer's glucostatic theory and renamed it "glucodynamic theory" as the glucose levels is not static but it is rather dynamic. As described above, glucose is the primary fuel of the brain thus it is critical to maintaining adequate glucose levels for appropriate brain function at all times. Blood glucose across the BBB *via* the saturable glucose transporter isoform 1. Brain glucose varies with blood glucose and is lower than that in the periphery [193]. In 1964, two groups suggested the presence of glucose-sensing neurons in the VMH (formerly known as satiety center) and LHA (formerly known as hunger center) using cats and dogs, respectively [194, 195]. Intravenous administration of glucose increased VHM- and reduced LHA-neuronal activities. Based on their responses to the extracellular glucose changes, these neurons are referred to glucose-excited (GE) or glucose-inhibited (GI) [196]. GE neurons are found in several hypothalamic areas, including ARC, VMN, PVN, and LHA (for review see [197]). As in pancreatic β-cell, increased central glucose activates glucokinase and raises ATP/ADP ratio leading to depolarization *via* closing ATP sensitive potassium channel (KATP) [198, 199]. In addition, neuronal glucose uptake is mediated by several glucose transporters such as GLUT3, GLUT2, and GLUT4 [200]. Kang *et al.* [200] have shown that insulin receptor is expressed in GE neurons. These glucose-sensing neurons have been shown to interact with several (an)orexigenic hypothalamic neuropeptides [201 - 204].

Recent study has shown that glucose levels in the cerebrospinal fluid (CSF) change proportionally to variations in blood glucose concentrations [205], suggesting the presence of a mechanism that transfers glucose from the blood to CSF. Further studies on primates and rodents showed that GLUTs, glucokinase (GK), and regulatory glucokinase protein (GKRP) are expressed in hypothalamic tanycytes [206, 207]. This indicates that tanycytes sense glucose variations in CSF and allow the incorporation of glucose from CSF to hypothalamic neuronal nuclei (Fig. **4.5**). Genetic inhibition of monocarbohydrate transporter, GLUT2, or GK in tanycytes showed impeded neuronal response to fasting and acute glucose administration, dysregulated expression of (an)orexigenic neuropeptides, and altered feeding behavior [208 - 210]. A recent study has shown that glucose activated sweet taste receptors and increased $Ca^{2+}$ concentrations in tanycytes (Fig. **4.5**) [211].

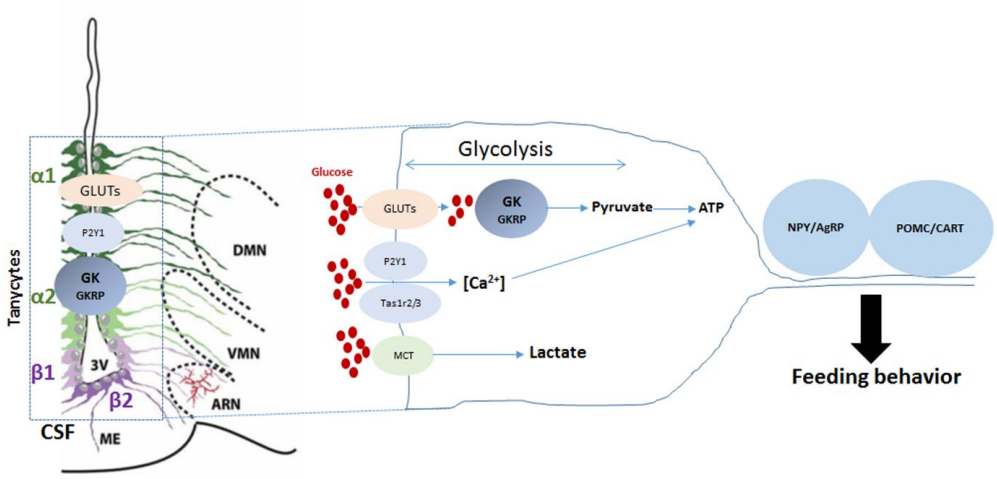


**Fig. (4.5). Model and potential mechanisms of glucose sensing mediated by tanycytes**. Tanycytes (α1, α2, β1, and β2) are distributed over the wall of the third ventricle (3V). The tanycytes have projections to hypothalamic neuronal nuclei such as VMN and ARC, and are joined by tight junctions forming part of ME-CSF barrier. Tanycytes sense CSF glucose variations *via* GLUTs, GK, Tas1r2/3, and MCT and allow incorporation of glucose to hypothalamic nuclei. Glucose can also generate metabolites like lactate. Inhibition of tanycyte GLUT, MCT, or GK alters the expression of hypothalamic (an)orexigenic neuropeptides and feeding behavior. AgRP, agouti regulated protein; ARC or ARN, arcuate nucleus; ATP, adenosine triphosphate; CART, cocaine and amphetamine-regulated transcript; CSF, cerebrospinal fluid; DMN, dorsomedial nucleus; GLUTs, glucose transporters; GK, glucokinase, GKRP, glucokinase regulated protein; MCT, monocarbohydrate transporter; Tas1r2/3, sweet taste receptors; VMN, ventromedial nucleus.

### *4.2.5. Hypothalamic Proteins and Amino Acids*

In 1956, Mellinkoff and colleagues showed a relationship between serum amino acid concentration and appetite and thereby advanced the a monostatic theory [212]. Several succeeding studies showed key roles for proteins in the regulation of appetite, food intake, body weight, and body composition. The satiety effect of proteins is source-dependent in both humans and animals [213], and has been demonstrated using deficient- or excess-protein diets. For instance, when rodents were fed a high protein diet with no other choice, they ate less compared with a regular diet containing a standard amount of proteins [214]. Similarly, when given a choice of 2 diets (high and low $CP^9$), rodents balance their dietary and protein intake [215]. Due to its suppressive effect on food intake, high protein diets became very popular and promoted weight loss [216].

Similar effects were observed in livestock. For instance, in both meat (broilers)- and egg (layer)-type chickens, reduced dietary protein content has been shown to induce feed intake [217, 218]. Pigs also eat more diets that are moderately deficient in CP or amino acids [219]. The association between diet CP content and dry matter intake has also been found in ruminants (for review see [220]).

The variety of proteins in a diet, with different amino acid sequences, digestion kinetics, and bioactive peptides, might explain their differential metabolic and physiological responses. For example, whey proteins have rapid and stronger satiety effects compared to casein [221].

Because the levels of brain and plasma amino acids rise relatively later after protein intake [222], it is likely that the satiety signal starts in the gastrointestinal tract (GI). Among the mechanisms involved in the satiety effects of proteins are:

1. Low palatability of proteins and generation of pre-absorptive signals while they are still in the GI. It has been shown that proteins trigger the release of intestinal hormones such as cholecystokinin (CCK) [223] by mucosal enteroendocrine cells, and evidence exists that CCK inhibits food intake [224].
2. Proteins have a greater thermogenic effect compared to other macronutrients [225].
3. Proteins induced gluconeogenesis[10] to prevent a decrease in glucose levels thereby contributing to satiety [226].
4. Interconnection between plasma and brain amino acid and central nutrient chemosensor system [227].

From a mechanistic standpoint, amino acids enter the brain *via* several facilitative carriers and amino acid transporter systems that are expressed in the BBB endothelial cells [228]. In addition, an amino-acid taste receptor (Tas1r1/Tas1r3) and umami taste receptor (mGLuR4) were detected in tanycytes, indicating a key role for tanycytes in amino acid sensing and detection (Fig. **4.6**).

Reports of anorexia-associated with indispensable amino acids (IAAs) deficiency in animal models dated from 1990 [229], and seemed to be orchestrated by the CNS [230]. As free amino acids are not stored like a carbohydrate (glycogen) or lipid (triglycerides), it was hypothesized that the decreased appetite associated with IAA deficiency is a protective mechanism to minimize any deleterious effect of disproportional IAA diets [231]. Tews *et al.* [232] used a branched-chain amino acid analog, norleucine, which competes with BCAA at the BBB, and showed a typical IAA deficiency-induced anorexia. Further lesioning studies suggested that the anterior piriform cortex (APC) is the primary IAA chemosensor [233]. Sharp *et al.* (reviewed in [227]) reported that mitogen-activated protein kinase (MAPK) was involved in mediating the anorectic effects of IAA deficiency. Magrum *et al.* [234] have shown, on the other hand, that central intracellular calcium concentration played a key role in the initial signal leading to alterations in neurotransmitters activity and food intake depression. Beverly and colleagues showed, by using inhibitor injection in the APC, a crucial role for intact RNA and protein synthesis in mediating the anorectic effects of IAA

deficiency [235]. Microdialysis studies demonstrated a consistent increase in BCAA concentration, but not the other amino acids with the exception of methionine and tyrosine, in the rodent LHA and PVN within 20-40 min after ingestion of a balanced amino acid mix or 50% protein meal [236].

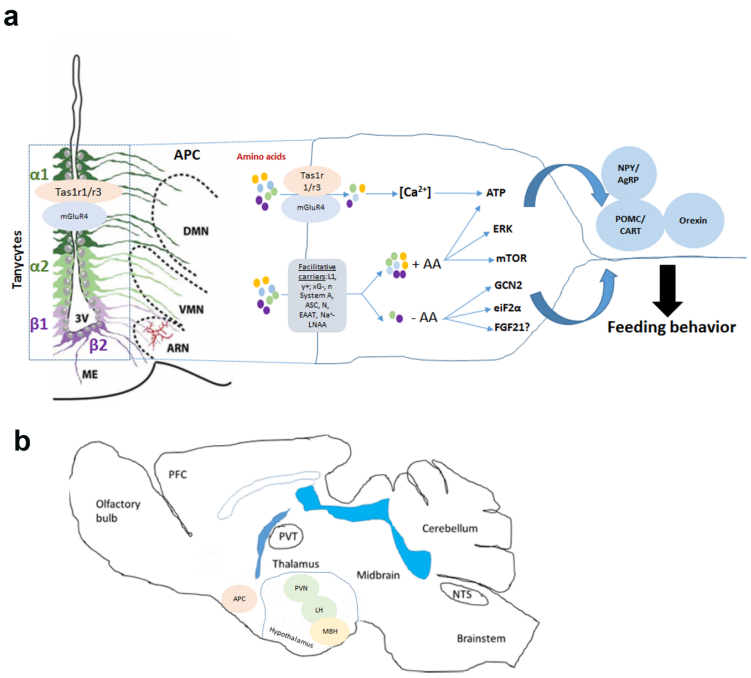


**Fig. (4.6). Model and potential mechanisms of central amino acid sensing and detection in feeding behavior. (a)** Amino acids enter the brain *via* facilitative carriers in the luminal and parenchymal membrane (system L1, $y^+$, $xG^-$, and n for large essential neutral AA, cationic AA, acidic AA, and glutamine, respectively). Other AA transporters systems such as system A (for small nonessential neutral AA, alanine), ASC (for some large and small neutral AA), N (for nitrogen-rich AA), EAAT (for the excitatory acidic AA), and Na+-LNAA (for large neutral AA) were also identified in the parenchymal membrane. Sensing protein excess regulates feeding behavior *via* ATP, mTOR, and ERK signaling pathways. Sensing protein deficit regulates feeding behavior through GCN2 and eiF2α. **(b)** schematic section of brain regions involved in amino acid sensing: APC senses amino acid imbalanced diets or very low-protein diets, NTS senses increased amino acid concentrations, MBH is involved in sensing bidirectional changes in amino acids, PVN and LH are involved in neuro-circuitory of amino acid sensing. The representation is not for scale. APC, anterior piriform cortex; ATP, adenosine triphosphate; eiF2α, Eukaryotic Translation Initiation Factor 2 Subunit Alpha; ERK, extracellular regulated kinase; GCN2, general control of amino-acid synthesis; LH, lateral hypothalamus; MBH, mediobasal hypothalamus; mTOR, mechanistic target of rapamycin; NTS, nucleus tractus solitaries; PVN, paraventricular nucleus.

At the molecular level, the detection of IAA-deficient diet within the APC has been shown to occur through general control of amino-acid synthesis 2 (GCN2)-dependent mechanism in both rodent and drosophila [237 - 239]. GCN2 is a serine/threonine-protein kinase that senses amino acid deficiency through binding

to uncharged tRNA [240]. Early work from Panksepp and booth in 1971 has shown that central administration of a balanced mixture of amino acid reduces food intake [241], suggesting a hypothalamic amino acid sensing mechanism. Subsequently, several studies have shown that ICV administration of leucine induces a hyperphagic response [242 - 244]. This anorectic effect is not produced by other BCAAs or any aromatic amino acid. In 2006, Seeley's group demonstrated that activation of the mechanistic target of rapamycin (mTOR) was associated with central leucine sensing in the regulation of feeding behavior [245]. The serine/threonine-protein kinase mTOR, a member of the phosphatidylinositol 3-kinase (PI3K)-kinase-related kinase superfamily, couples nutrient and growth factor sensing in the control of protein synthesis, growth, cell survival, and other cellular processes [246]. Injection of leucine phosphorylated ribosomal p70 S6 kinase 1 (S6k1), a major downstream mediator of mTOR pathway [247]. mTOR has been shown to be co-localized with hypothalamic feeding-related neuropeptides, and leucine was found to modulate the hypothalamic expression of AgRP [248]. Heeley and Blouet [249], however, suggested that mTOR signaling alone is not sufficient to explain the anorectic effect of leucine and hypothesized that the intracellular leucine metabolism and ATP production might play a role. Indeed, leucine produces α-ketoisocaproic acid (KIC) and isovaleryl-CoA *via* BCAA transferase (BCAT) and branched-chain ketoacid dehydrogenase (BCKDH), and KIC administration has been reported to suppress food intake in rodents [242]. Further studies have shown crosstalk between mTOR and GCN2 and their potential interaction with fibroblast growth factor 21(FGF21) in regulating food choice and metabolism [250 - 252] **(**Fig. **4.6).**

### *4.2.6. Non-coding RNAs*

Non-coding RNAs (ncRNAs) are functional RNA molecules that are transcribed from DNA but not translated into protein. Transfer RNA and ribosomal RNA were the first to be discovered in the 1950s. Other functional ncRNAs such as RNAse P, snRNAs, and 7SL were identified in the early 1980s. In 1993, Lee and colleagues made a breakthrough by discovering microRNAs (miRNAs) in the nematodes (*Caenorhabditis elegans*) [253]. The period that followed was marked by an inundate of information wherein several teams characterized numerous miRNAs from various species.

Based on current knowledge, DNA regions capable of generating mature functional miRNA can be present in diverse locations within the genome (introns, exons). An overview of miRNA biogenesis stepwise is illustrated in Fig. (**4.7**). Briefly, Initial nuclear processing of miRNA transcripts involves the microprocessor complex, which contains Drosha and DiGeorge syndrome critical

region gene 8 (DGCR8), also known as Pasha [254, 255]. This microprocessor cleaves pri-miRNA into pre-miRNA, which is transported into the cytoplasm by exportin 5, and where it is further processed into mature ~18- to 23-nucleotid--long duplexes by DICER1, with help from dsRNA-binding proteins, protein kinase RNA activator, and transactivation response RNA binding protein (TRBP) [256]. One strand of the dsRNA duplex is then loaded into an Argonaute (Ago) protein and drives the recruitment of a complex of effector proteins called the RNA-induced silencing complex (RISC) that inhibits the expression of targeted transcripts [257, 258].

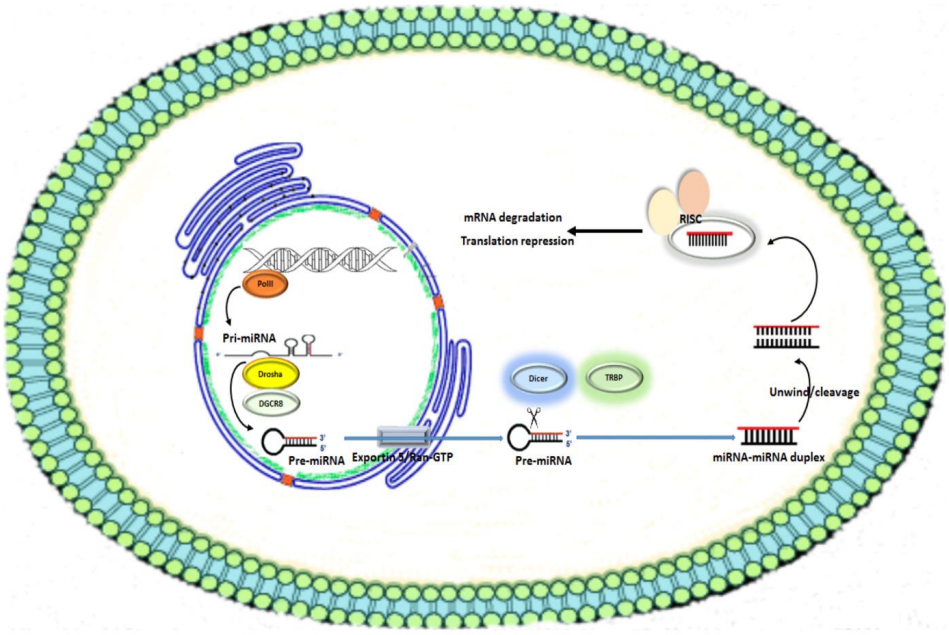


**Fig. (4.7). Schematic representation of miRNA biogenesis.** After transcription by RNA polymerase II, miRNA primary transcripts (pri-miRNAs) are cleaved by the microprocessor complex (Drosha/DGCR8) to produce a ~70 nucleotide precursor hairpin pre-miRNA in the nuclear compartment. The pre-miRNAs are then transported, *via* exportin 5, to the cytoplasm, where they are excised by dicer1 to form mature 22-nt miRNAs. One strand is selected for stable association with Argonaute, where it serves, in coordination with RISC, as a guide to target and regulate specific mRNAs. DGCR8, DiGeorge Syndrome Critical Region gene 8; RISC, RNA-induced silencing complex, TRBP, transactivation-responsive RNA binding protein.

The brain is considered a major site for miRNA expression, and miRNAs are key regulators for neurodevelopment, neurotransmission, and synaptic plasticity [259]. An alteration of miRNA machinery expression with an up-regulation of RISC genes (DGCR8 and Ago2) was found in the hypothalamus of the *anx/anx* anorexia mouse model [260], suggesting a potential role in the regulation of energy homeostasis. Interestingly, Claret's group has shown that more than 90%

of hypothalamic POMC and NPY/AgRP neurons express DICER1, which is modulated by fasting or overeating [261]. Furthermore, neuron-specific deletion of DICER1 induced hyperphagia and obesity in rodents [220, 262]. The effect of Dicer deletion/mutation seemed to be mediated by the activation of PI3K, AKT, and mTOR and loss of mir-103 [262].

Several studies revealed enrichment of miRNAs in the ARC and PVN of rodents and the preferential expression of miR-7a in NPY/AgRP neurons [263, 264]. Overexpression of miR-200a in the hypothalamus of ob/ob mice down-regulated the hypothalamic expression of insulin receptor substrate-2 (IRS2) and leptin receptor (Ob-R), which are key regulators of feeding behavior [265]. Sangiao-Alvarellos and colleagues demonstrated an alteration of hypothalamic let7a, mir-9, mir-30e, mir-132, mir-145, mir-200a, and mir-218 in high fat diet-fed rats and after chronic caloric restriction [266]. Based on their predicted targets, the effects of the abovementioned miRNAs were probably mediated *via* PI3K, AKT, insulin receptor (IR), P70S6K, JAK/STAT [266]. Using *in situ* hybridization, Derghal and co-workers, on the other hand, have shown that mir-383, mir-384-3p, and mir-488 are expressed in the POMC neurons and potentially can bind the 3-UTR of POMC mRNA [267]. The same group reported that the expression of these miRNAs was upregulated in the hypothalamus of ob/ob and db/db mice [267]. ICV administration of leptin down-regulated the hypothalamic expression of these miRNAs in both wild-type and obese (ob/ob) mice [267]. Recently, it has been shown that DICER1 is a prerequisite for food deprivation-induced autophagy in primary cortical neurons, partly *via* let-7 [268], suggesting a key role of autophagy in the regulation of food intake (see next section 4.2.7). Altogether, these studies showed that miRNA machinery plays a key role in the central regulation of energy homeostasis.

### *4.2.7. Autophagy*

#### *4.2.7.1. Autophagy Machinery*

Autophagy is an evolutionary preserved intracellular self-eating or self-digestion mechanism, whereby double-membrane autophagosome cloisters organelles or cytosol portions and delivers them to lysosomes for breakdown by resident hydrolases to provide nutrients and energy to the starved cells [269, 270].

Although the final destination of autophagy is the delivery of cargo to the lysosome for degradation and recycling, there are three primary types of autophagy:

1- Microautophagy: where cargos are captured by invaginations or protrusions of the lysosomal membrane [271]. The uptake occurs directly at the limiting membrane of the lysosome, and can include intact organelles.
2- Chaperone-mediated autophagy: where chaperones are used to identify cargo proteins with particular pentapeptide motif to unfold and translocate them individually directly across the lysosome membrane [272].
3- Macroautophagy: where a *de novo* synthesized double-membrane vesicles, called the autophagosome, are used to sequester cargo and subsequently transport it to the lysosome [273].

The macroautophagy (autophagy) process contains more than 30 genes that function in key stages of the pathway: initiation (or induction and nucleation), elongation, closure and maturation, fusion with the lysosomes, and degradation are shown in Fig. (**4.8**). During the initiation, the membrane is expanded to form a phagophore, which is the primary double-membrane sequestering compartment. Several studies indicated that plasma membrane, endoplasmic reticulum (ER), mitochondria, and Golgi apparatus are possible sources of the phagophore [274 - 276]. In mammalian cells, initiation and nucleation of the phagophore is controlled by ULK1/2, ATG13, C12orf44/ATG101, and RB1-inducible coiled-coil 1 (RB1CC1/FIP200) [277]. Under food-deprivation conditions, mTOR1 dissociates from the abovementioned induction complex, leading to partial dephosphorylation of ULK1/2 and ATG13, activating them and inducing autophagy. The nucleation stage is controlled by the ATG14-containing class III phosphatidylinositol 3 kinase (PtdIns3K) complex, which consists of PIK3C3/VPS34, PIK3R4/p150, BECN1, ATG14, and UVRAG (for review see [278]). There are two conjugation systems involving ubiquitin-like (UBL) proteins that contribute to the phagophore elongation. The first one contains ATG12-ATG5-ATG16L1 complex, and the second system is composed of ATG8-LC3 complex [279, 280]. In order to form a complete autophagosome, the phagophore must mature and close. The autophagosome traffics and fuse with endosome and/or lysosome, and this movement is dependent on microtubules. VTI1B, UVRAG, and SNARE machinery (VAM7/9 and syntaxin) have been reported to have a role in this fusion process [281 - 283].

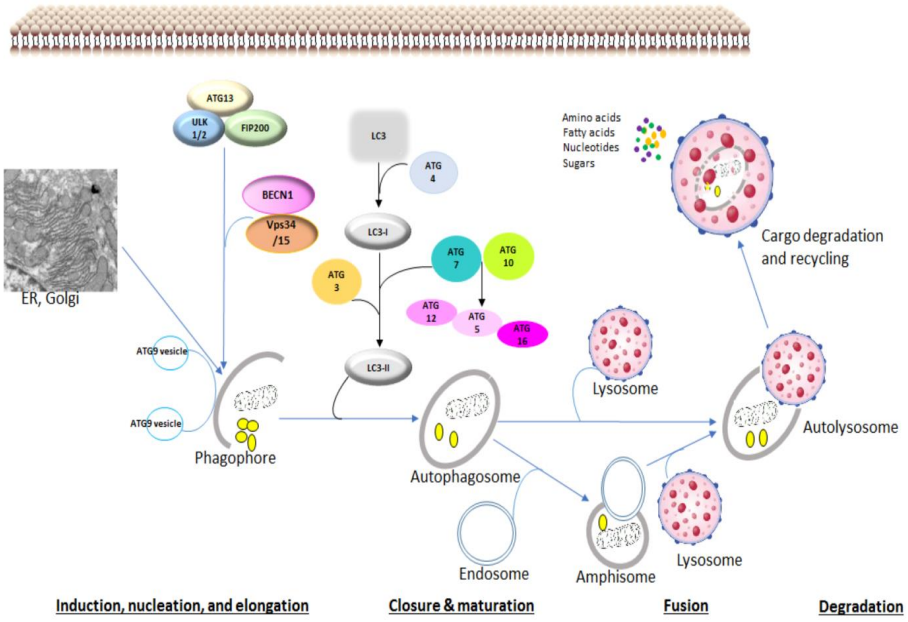


**Fig. (4.8). A schematic representation of autophagy machinery process**. The sequential process of autophagy consists of induction, nucleation, and elongation of the phagophore, followed by the formation of autophagosome and amphisome, which in turn, fuse with a lysosome to form autolysosome that leads to cargo degradation and recycling of macromolecules. These stages and processes are controlled by different sets of autophagy-related genes (ATGs). ATG, autophagy-related gene; BECN1, beclin 1; FIP200, family kinase-interacting protein of 200 kDa; LC3, microtubule-associated protein 1A/1B light chain 3; ULK, Unc-51 like autophagy activating kinase; VPS, vacuolar protein sorting.

### *4.2.7.2. Autophagy and Food Intake*

It has been hypothesized that hypothalamic autophagy is nutrient-responsive and that the induction of autophagy in the hypothalamus during food deprivation mobilizes neuron-intrinsic lipids to generate free fatty acids that control food intake [284]. The same group demonstrated that fasting activated autophagy in both rodent MBH and GT1-7 cell culture [285]. Specifically, they showed that fasting increased hypothalamic fatty acid uptake and suggested that the source of central lipids during food deprivation is the periphery [285]. Exposure of hypothalamic GT1-7 cells to fatty acids (OA and PA) induced AMPK and ULK1 phosphorylated levels and activated autophagy [285]. Specific knock-out of ATG7 in AgRP neurons, orexigenic cell types, significantly reduced food intake in mice upon refeeding after 6 or 24h fast [285]. These changes were accompanied by an increased expression of hypothalamic α-MSH. In contrast, Meng and Cai [286] found that chronic high-fat diet and obesity were associated

with reduced hypothalamic ATG7 expression. Furthermore, they showed that genetic knockdown of ATG7 resulted in increased food intake and obesity potentially *via* NF-KB-mediated inflammation [286]. Xiao and co-workers have shown that hypothalamic activating transcription factor 4 (ATF4)/ATG5 axis in POMC neurons play a key role in the regulation of energy balance (intake and expenditure) [287]. Interestingly, Malhotra *et al.* [288] have shown that loss of ATG12, but not ATG5, in POMC neurons increased food intake and accelerated weight gain and adiposity. Together these elegant studies demonstrated a key role of autophagy in the regulation of energy homeostasis and raised more questions that open new research vistas. For instance, what other hypothalamic ATGs are involved in the regulation of energy balance? Do the manipulation of these ATG in specific neurons have specific outcomes? Similar to fatty acids, does hypothalamic autophagy glucose- or amino acid-responsive? What are the upstream regulators of hypothalamic autophagy?

### *4.2.8. Hypothalamic Mitochondrial Mitofusin 2*

Mitofusin 2 (MFN2) plays a critical role in both mitochondrial dynamic (fusion) and the establishment of mitochondria-ER interactions. Claret's group has shown that POMC-specific knock down of MFN2 resulted in the loss of mitochondria-ER contacts, induced hyperphagia, reduced energy expenditure, and induced obesity and leptin resistance [289]. MFN2 overexpression in the ARC reduced food intake, body weight, adiposity, and plasma leptin levels in diet-induced obese (DIO) mice [289]. The study unraveled a pivotal role of mitochondria network and mitochondria-ER contact in regulating hypothalamic POMC neuronal function and whole-body energy homeostasis.

## CONCLUSION

Classical and new information regarding central neuronal circuits that regulate appetite and food intake has extended our understanding of energy balance. Many research groups and seminal works broke through by identifying several hypothalamic signaling pathways (orexigenic and anorexigenic) that regulate food intake and body weight and constitute potential therapeutic targets to treat metabolic and feeding-related disorders.

## NOTES

[1] Hyperphagia is an abnormal condition of intense hunger and excessive eating

[2] Aphagia: passive aphagia when an animal does not respond to food if it is

presented; active aphagia when an animal rejects the food; and mixed aphagia when an animal does not react to food when presented, but spit it out when is placed in the mouth.

[3] Orexigenic: appetite stimulator

[4] Anorexigenic: appetite inhibitor

[5] ICV, intracerebroventricular

[6] ICV, intracerebroventricular

[7] IN, infindibular nucleus

[8] KO mice, knock out mice

[9] CP: crude proteins

[10] Gluconeogenesis is a metabolic pathway resulting in the generation of glucose from non-carbohydrate substrates

## REFERENCES

[1] Brobeck JR, Tepperman J, Long CN. Experimental Hypothalamic Hyperphagia in the Albino Rat. Yale J Biol Med 1943; 15(6): 831-53.
[PMID: 21434115]

[2] Brooks CM, Lockwood RA, Wiggins ML. A study of the effect of hypothalamic lesions on the eating habits of the albino rat. Am J Physiol 1946; 147(4): 735-41.
[http://dx.doi.org/10.1152/ajplegacy.1946.147.4.735] [PMID: 20277066]

[3] Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. Yale J Biol Med 1951; 24(2): 123-40.
[PMID: 14901884]

[4] Kuenzel WJ. Central neuroanatomical systems involved in the regulation of food intake in birds and mammals. J Nutr 1994; 124(8) (Suppl. 8): 1355S-70S.
[http://dx.doi.org/10.1093/jn/124.suppl_8.1355S] [PMID: 8064384]

[5] Woods SC, Seeley RJ, Porte D Jr, Schwartz MW. Signals that regulate food intake and energy homeostasis. Science 1998; 280(5368): 1378-83.
[http://dx.doi.org/10.1126/science.280.5368.1378] [PMID: 9603721]

[6] Miner JL, Della-Fera MA, Paterson JA, Baile CA. Lateral cerebroventricular injection of neuropeptide Y stimulates feeding in sheep. Am J Physiol 1989; 257(2 Pt 2): R383-7.
[PMID: 2764161]

[7] Kuenzel WJ, Douglass LW, Davison BA. Robust feeding following central administration of neuropeptide Y or peptide YY in chicks, Gallus domesticus. Peptides 1987; 8(5): 823-8.
[http://dx.doi.org/10.1016/0196-9781(87)90066-0] [PMID: 3432131]

[8] Schick RR, Schusdziarra V, Nussbaumer C, Classen M. Neuropeptide Y and food intake in fasted rats: effect of naloxone and site of action. Brain Res 1991; 552(2): 232-9.
[http://dx.doi.org/10.1016/0006-8993(91)90087-C] [PMID: 1913187]

[9] Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology 1993; 133(4): 1753-8.
[http://dx.doi.org/10.1210/endo.133.4.8404618] [PMID: 8404618]

[10] Glenn Stanley B, Kyrkouli SE, Lampert S, Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. Peptides 1986; 7(6): 1189-92.
[http://dx.doi.org/10.1016/0196-9781(86)90149-X] [PMID: 3470711]

[11] Gerald C, Walker MW, Criscione L, *et al.* A receptor subtype involved in neuropeptide-Y-induced food intake. Nature 1996; 382(6587): 168-71.
[http://dx.doi.org/10.1038/382168a0] [PMID: 8700207]

[12] Kuenzel WJ, Hancock M, Nagarajan G, Aman NA, Kang SW. Central effect of vasotocin 4 receptor (VT4R/V1aR) antagonists on the stress response and food intake in chicks given neuropeptide Y (NPY). Neurosci Lett 2016; 620: 57-61.
[http://dx.doi.org/10.1016/j.neulet.2016.03.036] [PMID: 27016389]

[13] Bungo T, Ando R, Kawakami SI, *et al.* Central bombesin inhibits food intake and the orexigenic effect of neuropeptide Y in the neonatal chick. Physiol Behav 2000; 70(5): 573-6.
[http://dx.doi.org/10.1016/S0031-9384(00)00301-2] [PMID: 11111013]

[14] Nelson LA, Gilbert ER, Cline MA. Effects of dietary macronutrient composition on exogenous neuropeptide Y's stimulation of food intake in chicks. Neurosci Lett 2015; 591: 171-5.
[http://dx.doi.org/10.1016/j.neulet.2015.02.017] [PMID: 25681626]

[15] Strader AD, Buntin JD. Neuropeptide-Y: a possible mediator of prolactin-induced feeding and regulator of energy balance in the ring dove (Streptopelia risoria). J Neuroendocrinol 2001; 13(4): 386-92.
[http://dx.doi.org/10.1046/j.1365-2826.2001.00642.x] [PMID: 11264727]

[16] Richardson RD, Boswell T, Raffety BD, Seeley RJ, Wingfield JC, Woods SC. NPY increases food intake in white-crowned sparrows: effect in short and long photoperiods. Am J Physiol 1995; 268(6 Pt 2): R1418-22.
[PMID: 7611518]

[17] Denbow DM, Duke GE, Chaplin SB. Food intake, gastric secretion, and motility as affected by avian pancreatic polypeptide administered centrally in chickens. Peptides 1988; 9(3): 449-54.
[http://dx.doi.org/10.1016/0196-9781(88)90146-5] [PMID: 3420004]

[18] Tachibana T, Sugahara K, Ohgushi A, *et al.* Intracerebroventricular injection of agouti-related protein attenuates the anorexigenic effect of alpha-melanocyte stimulating hormone in neonatal chicks. Neurosci Lett 2001; 305(2): 131-4.
[http://dx.doi.org/10.1016/S0304-3940(01)01827-4] [PMID: 11376901]

[19] Hagen CJ, Newmyer BA, Webster RI, *et al.* Stimulation of food intake after central galanin is associated with arcuate nucleus activation and does not differ between genetically selected low and high body weight lines of chickens. Neuropeptides 2013; 47(4): 281-5.
[http://dx.doi.org/10.1016/j.npep.2012.11.003] [PMID: 23369300]

[20] Tachibana T, Cline MA, Sugahara K, Ueda H, Hiramatsu K. Central administration of somatostatin stimulates feeding behavior in chicks. Gen Comp Endocrinol 2009; 161(3): 354-9.

[http://dx.doi.org/10.1016/j.ygcen.2009.01.022] [PMID: 19523380]

[21] Cline MA, Calchary WA, Nandar W. Effect of calcitonin gene-related peptide (CGRP) on avian appetite-related processes. Behav Brain Res 2009; 196(2): 242-7.
[http://dx.doi.org/10.1016/j.bbr.2008.09.004] [PMID: 18835571]

[22] Tachibana T, Takagi T, Tomonaga S, *et al.* Central administration of cocaine- and amphetamine-regulated transcript inhibits food intake in chicks. Neurosci Lett 2003; 337(3): 131-4.
[http://dx.doi.org/10.1016/S0304-3940(02)01321-6] [PMID: 12536041]

[23] Cline MA, Nandar W, Rogers JO. Central neuropeptide FF reduces feed consumption and affects hypothalamic chemistry in chicks. Neuropeptides 2007; 41(6): 433-9.
[http://dx.doi.org/10.1016/j.npep.2007.08.003] [PMID: 17936900]

[24] Prall BC, Cline MA. Anorexigenic effects of central neuropeptide K are associated with hypothalamic changes in juvenile Gallus gallus. Gen Comp Endocrinol 2008; 159(2-3): 130-5.
[http://dx.doi.org/10.1016/j.ygcen.2008.08.007] [PMID: 18786538]

[25] Masunari K, Khan MSI, Cline MA, Tachibana T. Central administration of mesotocin inhibits feeding behavior in chicks. Regul Pept 2013; 187: 1-5.
[http://dx.doi.org/10.1016/j.regpep.2013.10.004] [PMID: 24183984]

[26] Tachibana T, Khan MSI, Matsuda K, Ueda H, Cline MA. Central administration of substance P inhibits feeding behavior in chicks. Horm Behav 2010; 57(2): 203-8.
[http://dx.doi.org/10.1016/j.yhbeh.2009.11.001] [PMID: 19909753]

[27] Tachibana T, Sato M, Takahashi H, Ukena K, Tsutsui K, Furuse M. Gonadotropin-inhibiting hormone stimulates feeding behavior in chicks. Brain Res 2005; 1050(1-2): 94-100.
[http://dx.doi.org/10.1016/j.brainres.2005.05.035] [PMID: 15979587]

[28] Ukena K, Tachibana T, Iwakoshi-Ukena E, *et al.* Identification, localization, and function of a novel avian hypothalamic neuropeptide, 26RFa, and its cognate receptor, G protein-coupled receptor-103. Endocrinology 2010; 151(5): 2255-64.
[http://dx.doi.org/10.1210/en.2009-1478] [PMID: 20308530]

[29] Tachibana T, Saito S, Tomonaga S, *et al.* Effect of central administration of prolactin-releasing peptide on feeding in chicks. Physiol Behav 2004; 80(5): 713-9.
[http://dx.doi.org/10.1016/j.physbeh.2003.12.005] [PMID: 14984806]

[30] Shipp SL, Yi J, Dridi S, Gilbert ER, Cline MA. The central anorexigenic mechanism of adrenocorticotropic hormone involves the caudal hypothalamus in chicks. Neuropeptides 2015; 53: 29-35.
[http://dx.doi.org/10.1016/j.npep.2015.07.005] [PMID: 26297349]

[31] Kawakami S, Bungo T, Ando R, *et al.* Central administration of α-melanocyte stimulating hormone inhibits fasting- and neuropeptide Y-induced feeding in neonatal chicks. Eur J Pharmacol 2000; 398(3): 361-4.
[http://dx.doi.org/10.1016/S0014-2999(00)00344-7] [PMID: 10862825]

[32] Denbow DM, Snapir N, Furuse M. Inhibition of food intake by CRF in chickens. Physiol Behav 1999; 66(4): 645-9.
[http://dx.doi.org/10.1016/S0031-9384(98)00340-0] [PMID: 10386909]

[33] Zhang R, Nakanishi T, Ohgushi A, *et al.* Suppression of food intake induced by corticotropin-releasing factor family in neonatal chicks. Eur J Pharmacol 2001; 427(1): 37-41.
[http://dx.doi.org/10.1016/S0014-2999(01)01109-8] [PMID: 11553361]

[34] Ogino M, Okumura A, Khan MSI, Cline MA, Tachibana T. Comparison of brain urocortin-3 and corticotrophin-releasing factor for physiological responses in chicks. Physiol Behav 2014; 125: 57-61.
[http://dx.doi.org/10.1016/j.physbeh.2013.11.006] [PMID: 24291384]

[35] Cline MA, Prall BC, Rogers JO, Tachibana T. Satiety induced by central stresscopin is mediated by corticotrophin-releasing factor receptors and hypothalamic changes in chicks. Pharmacol Biochem

Behav 2009; 92(4): 663-9.
[http://dx.doi.org/10.1016/j.pbb.2009.03.005] [PMID: 19324068]

[36] Furuse M, Matsumoto M, Okumura J, Sugahara K, Hasegawa S. Intracerebroventricular injection of mammalian and chicken glucagon-like peptide-1 inhibits food intake of the neonatal chick. Brain Res 1997; 755(1): 167-9.
[http://dx.doi.org/10.1016/S0006-8993(97)00190-X] [PMID: 9163555]

[37] Honda K, Saneyasu T, Yamaguchi T, *et al.* Intracerebroventricular administration of chicken oxyntomodulin suppresses food intake and increases plasma glucose and corticosterone concentrations in chicks. Neurosci Lett 2014; 564: 57-61.
[http://dx.doi.org/10.1016/j.neulet.2014.02.008] [PMID: 24530259]

[38] Tachibana T, Sugimoto I, Ogino M, *et al.* Central administration of chicken growth hormone-releasing hormone decreases food intake in chicks. Physiol Behav 2015; 139: 195-201.
[http://dx.doi.org/10.1016/j.physbeh.2014.11.043] [PMID: 25449398]

[39] Zhang X, Tang N, Qi J, *et al.* CCK reduces the food intake mainly through CCK1R in Siberian sturgeon (Acipenser baerii Brandt). Sci Rep 2017; 7(1): 12413.
[http://dx.doi.org/10.1038/s41598-017-12646-3] [PMID: 28963554]

[40] Furuse M, Ao R, Bungo T, *et al.* Central gastrin inhibits feeding behavior and food passage in neonatal chicks. Life Sci 1999; 65(3): 305-11.
[http://dx.doi.org/10.1016/S0024-3205(99)00249-0] [PMID: 10447216]

[41] Gibbs J, Smith GP. Satiety: the roles of peptides from the stomach and the intestine. Fed Proc 1986; 45(5): 1391-5.
[PMID: 3956760]

[42] Kamisoyama H, Honda K, Saneyasu T, Sugahara K, Hasegawa S. Central administration of neuromedin U suppresses food intake in chicks. Neurosci Lett 2007; 420(1): 1-5.
[http://dx.doi.org/10.1016/j.neulet.2007.03.062] [PMID: 17445984]

[43] Yanagita K, Shiraishi JI, Fujita M, Bungo T. Effects of N-terminal fragments of β-endorphin on feeding in chicks. Neurosci Lett 2008; 442(2): 140-2.
[http://dx.doi.org/10.1016/j.neulet.2008.07.018] [PMID: 18638524]

[44] Fichna J, Janecka A, Costentin J, Do Rego JC. The endomorphin system and its evolving neurophysiological role. Pharmacol Rev 2007; 59(1): 88-123.
[http://dx.doi.org/10.1124/pr.59.1.3] [PMID: 17329549]

[45] Bungo T, Shiraishi J, Yanagita K, Ohta Y, Fujita M. Effect of nociceptin/orphanin FQ on feeding behavior and hypothalamic neuropeptide expression in layer-type chicks. Gen Comp Endocrinol 2009; 163(1-2): 47-51.
[http://dx.doi.org/10.1016/j.ygcen.2009.03.009] [PMID: 19318106]

[46] Morley JE, Hernandez EN, Flood JF. Neuropeptide Y increases food intake in mice. Am J Physiol 1987; 253(3 Pt 2): R516-22.
[PMID: 3631310]

[47] Clark JT, Sahu A, Kalra PS, Balasubramaniam A, Kalra SP, Neuropeptide Y. Neuropeptide Y (NPY)-induced feeding behavior in female rats: comparison with human NPY ([Met17]NPY), NPY analog ([norLeu4]NPY) and peptide YY. Regul Pept 1987; 17(1): 31-9.
[http://dx.doi.org/10.1016/0167-0115(87)90030-9] [PMID: 3562905]

[48] Stanley BG, Leibowitz SF. Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. Proc Natl Acad Sci USA 1985; 82(11): 3940-3.
[http://dx.doi.org/10.1073/pnas.82.11.3940] [PMID: 3858854]

[49] McMahon CD, Buxton DF, Elsasser TH, *et al.* Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. J Endocrinol 1999; 161(2): 333-9.

[http://dx.doi.org/10.1677/joe.0.1610333] [PMID: 10320832]

[50] Taylor IL, Garcia R. Effects of pancreatic polypeptide, caerulein, and bombesin on satiety in obese mice. Am J Physiol 1985; 248(3 Pt 1): G277-80.
[PMID: 3976886]

[51] Ueno N, Inui A, Iwamoto M, *et al.* Decreased food intake and body weight in pancreatic polypeptide-overexpressing mice. Gastroenterology 1999; 117(6): 1427-32.
[http://dx.doi.org/10.1016/S0016-5085(99)70293-3] [PMID: 10579984]

[52] Halatchev IG, Ellacott KLJ, Fan W, Cone RD. Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. Endocrinology 2004; 145(6): 2585-90.
[http://dx.doi.org/10.1210/en.2003-1754] [PMID: 15016721]

[53] Batterham RL, Heffron H, Kapoor S, *et al.* Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab 2006; 4(3): 223-33.
[http://dx.doi.org/10.1016/j.cmet.2006.08.001] [PMID: 16950139]

[54] Day DE, Bartness TJ. Agouti-related protein increases food hoarding more than food intake in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 2004; 286(1): R38-45.
[http://dx.doi.org/10.1152/ajpregu.00284.2003] [PMID: 14500267]

[55] Karatayev O, Baylan J, Leibowitz SF. Increased intake of ethanol and dietary fat in galanin overexpressing mice. Alcohol 2009; 43(8): 571-80.
[http://dx.doi.org/10.1016/j.alcohol.2009.09.025] [PMID: 20004335]

[56] Stengel A, Coskun T, Goebel M, *et al.* Central injection of the stable somatostatin analog ODT8-SST induces a somatostatin2 receptor-mediated orexigenic effect: role of neuropeptide Y and opioid signaling pathways in rats. Endocrinology 2010; 151(9): 4224-35.
[http://dx.doi.org/10.1210/en.2010-0195] [PMID: 20610566]

[57] Nilsson C, Hansen TK, Rosenquist C, *et al.* Long acting analogue of the calcitonin gene-related peptide induces positive metabolic effects and secretion of the glucagon-like peptide-1. Eur J Pharmacol 2016; 773: 24-31.
[http://dx.doi.org/10.1016/j.ejphar.2016.01.003] [PMID: 26808305]

[58] Kristensen P, Judge ME, Thim L, *et al.* Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998; 393(6680): 72-6.
[http://dx.doi.org/10.1038/29993] [PMID: 9590691]

[59] Murase T, Arima H, Kondo K, Oiso Y. Neuropeptide FF reduces food intake in rats. Peptides 1996; 17(2): 353-4.
[http://dx.doi.org/10.1016/0196-9781(95)02137-X] [PMID: 8801545]

[60] Sahu A, Kalra PS, Dube MG, Kalra SP. Neuropeptide K suppresses feeding in the rat. Regul Pept 1988; 23(2): 135-43.
[http://dx.doi.org/10.1016/0167-0115(88)90021-3] [PMID: 3231743]

[61] Peng YL, Han RW, Chang M, *et al.* Central Neuropeptide S inhibits food intake in mice through activation of Neuropeptide S receptor. Peptides 2010; 31(12): 2259-63.
[http://dx.doi.org/10.1016/j.peptides.2010.08.015] [PMID: 20800637]

[62] Arletti R, Benelli A, Bertolini A. Oxytocin inhibits food and fluid intake in rats. Physiol Behav 1990; 48(6): 825-30.
[http://dx.doi.org/10.1016/0031-9384(90)90234-U] [PMID: 2087513]

[63] Karagiannides I, Torres D, Tseng YH, *et al.* Substance P as a novel anti-obesity target. Gastroenterology 2008; 134(3): 747-755.e1.
[http://dx.doi.org/10.1053/j.gastro.2007.12.032] [PMID: 18325388]

[64] Meyer AH, Langhans W, Scharrer E. Vasopressin reduces food intake in goats. Q J Exp Physiol 1989; 74(4): 465-73.
[http://dx.doi.org/10.1113/expphysiol.1989.sp003294] [PMID: 2798756]

[65] Clarke IJ, Smith JT, Henry BA, *et al.* Gonadotropin-inhibitory hormone is a hypothalamic peptide that provides a molecular switch between reproduction and feeding. Neuroendocrinology 2012; 95(4): 305-16.
[http://dx.doi.org/10.1159/000332822] [PMID: 22286004]

[66] Chartrel N, Dujardin C, Anouar Y, *et al.* Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. Proc Natl Acad Sci USA 2003; 100(25): 15247-52.
[http://dx.doi.org/10.1073/pnas.2434676100] [PMID: 14657341]

[67] Lawrence CB, Liu YL, Stock MJ, Luckman SM. Anorectic actions of prolactin-releasing peptide are mediated by corticotropin-releasing hormone receptors. Am J Physiol Regul Integr Comp Physiol 2004; 286(1): R101-7.
[http://dx.doi.org/10.1152/ajpregu.00402.2003] [PMID: 14512273]

[68] Vergoni AV, Poggioli R, Bertolini A. Corticotropin inhibits food intake in rats. Neuropeptides 1986; 7(2): 153-8.
[http://dx.doi.org/10.1016/0143-4179(86)90091-0] [PMID: 3010169]

[69] Dutia R, Meece K, Dighe S, Kim AJ, Wardlaw SL. β-Endorphin antagonizes the effects of α-MSH on food intake and body weight. Endocrinology 2012; 153(9): 4246-55.
[http://dx.doi.org/10.1210/en.2012-1166] [PMID: 22778225]

[70] George SA, Khan S, Briggs H, Abelson JL. CRH-stimulated cortisol release and food intake in healthy, non-obese adults. Psychoneuroendocrinology 2010; 35(4): 607-12.
[http://dx.doi.org/10.1016/j.psyneuen.2009.09.017] [PMID: 19828258]

[71] Negri L, Noviello L, Noviello V. Effects of sauvagine, urotensin I and CRF on food intake in rats. Peptides 1985; 6 (Suppl. 3): 53-7.
[http://dx.doi.org/10.1016/0196-9781(85)90350-X] [PMID: 3879535]

[72] Ushikai M, Asakawa A, Sakoguchi T, Tanaka C, Inui A. Centrally administered urocortin 3 inhibits food intake and gastric emptying in mice. Endocrine 2011; 39(2): 113-7.
[http://dx.doi.org/10.1007/s12020-010-9420-7] [PMID: 21061090]

[73] Fekete ÉM, Zorrilla EP. Physiology, pharmacology, and therapeutic relevance of urocortins in mammals: Ancient CRF paralogs. Front Neuroendocrinol 2007; 28(1): 1-27.
[http://dx.doi.org/10.1016/j.yfrne.2006.09.002] [PMID: 17083971]

[74] Turton MD, O'Shea D, Gunn I, *et al.* A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 1996; 379(6560): 69-72.
[http://dx.doi.org/10.1038/379069a0] [PMID: 8538742]

[75] Tang-Christensen M, Larsen PJ, Göke R, *et al.* Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. Am J Physiol 1996; 271(4 Pt 2): R848-56.
[PMID: 8897973]

[76] Dakin CL, Gunn I, Small CJ, *et al.* Oxyntomodulin inhibits food intake in the rat. Endocrinology 2001; 142(10): 4244-50.
[http://dx.doi.org/10.1210/endo.142.10.8430] [PMID: 11564680]

[77] Okada K, Ishii S, Minami S, Sugihara H, Shibasaki T, Wakabayashi I. Intracerebroventricular administration of the growth hormone-releasing peptide KP-102 increases food intake in free-feeding rats. Endocrinology 1996; 137(11): 5155-8.
[http://dx.doi.org/10.1210/endo.137.11.8895390] [PMID: 8895390]

[78] Gibbs J, Young RC, Smith GP. Cholecystokinin decreases food intake in rats. 1973. Obes Res 1997; 5(3): 284-90.
[http://dx.doi.org/10.1002/j.1550-8528.1997.tb00305.x] [PMID: 9192405]

[79] Perry B, Wang Y. Appetite regulation and weight control: the role of gut hormones. Nutr Diabetes 2012; 2(1): e26.
[http://dx.doi.org/10.1038/nutd.2011.21] [PMID: 23154682]

[80] Roesler R, Schwartsmann G. Gastrin-releasing peptide receptors in the central nervous system: role in brain function and as a drug target. Front Endocrinol (Lausanne) 2012; 3: 159. [http://dx.doi.org/10.3389/fendo.2012.00159] [PMID: 23251133]

[81] Ladenheim EE, Taylor JE, Coy DH, Moran TH. Blockade of feeding inhibition by neuromedin B using a selective receptor antagonist. Eur J Pharmacol 1994; 271(1): R7-9. [http://dx.doi.org/10.1016/0014-2999(94)90291-7] [PMID: 7698191]

[82] Margules DL, Moisset B, Lewis MJ, Shibuya H, Pert CB. β-Endorphin Is Associated with Overeating in Genetically Obese Mice ( *ob* / *ob* ) and Rats ( *fa* / *fa* ). Science 1978; 202(4371): 988-91. [http://dx.doi.org/10.1126/science.715455] [PMID: 715455]

[83] Olszewski P, Grace MK, Sanders JB, Billington CJ, Levine AS. Effect of nociceptin/orphanin FQ on food intake in rats that differ in diet preference. Pharmacol Biochem Behav 2002; 73(3): 529-35. [http://dx.doi.org/10.1016/S0091-3057(02)00821-3] [PMID: 12151026]

[84] Graham M, Shutter JR, Sarmiento U, Sarosi , Stark KL. Overexpression of Agrt leads to obesity in transgenic mice. Nat Genet 1997; 17(3): 273-4. [http://dx.doi.org/10.1038/ng1197-273] [PMID: 9354787]

[85] Huszar D, Lynch CA, Fairchild-Huntress V, *et al.* Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 1997; 88(1): 131-41. [http://dx.doi.org/10.1016/S0092-8674(00)81865-6] [PMID: 9019399]

[86] Kim MS, Rossi M, Abbott CR, AlAhmed SH, Smith DM, Bloom SR. Sustained orexigenic effect of Agouti related protein may be not mediated by the melanocortin 4 receptor. Peptides 2002; 23(6): 1069-76. [http://dx.doi.org/10.1016/S0196-9781(02)00039-6] [PMID: 12126733]

[87] Kim KS, Reecy JM, Hsu WH, Anderson LL, Rothschild MF. Functional and phylogenetic analyses of a melanocortin-4 receptor mutation in domestic pigs. Domest Anim Endocrinol 2004; 26(1): 75-86. [http://dx.doi.org/10.1016/j.domaniend.2003.12.001] [PMID: 14732454]

[88] Lewis DE, Shellard L, Koeslag DG, *et al.* Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. Am J Physiol 1993; 264(2 Pt 1): E279-84. [PMID: 8447395]

[89] Mizuno TM, Makimura H, Silverstein J, Roberts JL, Lopingco T, Mobbs CV. Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. Endocrinology 1999; 140(10): 4551-7. [http://dx.doi.org/10.1210/endo.140.10.6966] [PMID: 10499510]

[90] Mizuno TM, Mobbs CV. Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 1999; 140(2): 814-7. [http://dx.doi.org/10.1210/endo.140.2.6491] [PMID: 9927310]

[91] Yaswen L, Diehl N, Brennan MB, Hochgeschwender U. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nat Med 1999; 5(9): 1066-70. [http://dx.doi.org/10.1038/12506] [PMID: 10470087]

[92] Krude H, Biebermann H, Luck W, Horn R, Brabant G, Grüters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 1998; 19(2): 155-7. [http://dx.doi.org/10.1038/509] [PMID: 9620771]

[93] Chen AS, Marsh DJ, Trumbauer ME, *et al.* Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat Genet 2000; 26(1): 97-102. [http://dx.doi.org/10.1038/79254] [PMID: 10973258]

[94] McMinn JE, Wilkinson CW, Havel PJ, Woods SC, Schwartz MW. Effect of intracerebroventricular α-MSH on food intake, adiposity, c-Fos induction, and neuropeptide expression. Am J Physiol Regul Integr Comp Physiol 2000; 279(2): R695-703.

[http://dx.doi.org/10.1152/ajpregu.2000.279.2.R695] [PMID: 10938261]

[95] Saneyasu T, Honda K, Kamisoyama H, Nakayama Y, Ikegami K, Hasegawa S. Alpha-melanocyte stimulating hormone plays an important role in the regulation of food intake by the central melanocortin system in chicks. Peptides 2011; 32(5): 996-1000.
[http://dx.doi.org/10.1016/j.peptides.2011.03.006] [PMID: 21402117]

[96] Harrold JA, Widdowson PS, Williams G. β-MSH: a functional ligand that regulated energy homeostasis via hypothalamic MC4-R? Peptides 2003; 24(3): 397-405.
[http://dx.doi.org/10.1016/S0196-9781(03)00054-8] [PMID: 12732337]

[97] Ling MK, Hotta E, Kilianova Z, *et al.* The melanocortin receptor subtypes in chicken have high preference to ACTH-derived peptides. Br J Pharmacol 2004; 143(5): 626-37.
[http://dx.doi.org/10.1038/sj.bjp.0705900] [PMID: 15466451]

[98] Li HY, Hwang HW, Hu YH. Functional characterizations of cocaine- and amphetamine-regulated transcript mRNA expression in rat hypothalamus. Neurosci Lett 2002; 323(3): 203-6.
[http://dx.doi.org/10.1016/S0304-3940(02)00151-9] [PMID: 11959420]

[99] Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Mol Endocrinol 1994; 8(10): 1298-308.
[PMID: 7854347]

[100] Carvajal F, Lerma-Cabrera JM, Alcaraz-Iborra M, Navarro M, Thiele TE, Cubero I. Nucleus Accumbens MC4-R Stimulation Reduces Food and Ethanol Intake in Adult Rats Regardless of Binge-Like Ethanol Exposure during Adolescence. Front Behav Neurosci 2017; 11: 167.
[http://dx.doi.org/10.3389/fnbeh.2017.00167] [PMID: 28936166]

[101] Butler AA. The melanocortin system and energy balance. Peptides 2006; 27(2): 281-90.
[http://dx.doi.org/10.1016/j.peptides.2005.02.029] [PMID: 16434123]

[102] Sutton GM, Trevaskis JL, Hulver MW, *et al.* Diet-genotype interactions in the development of the obese, insulin-resistant phenotype of C57BL/6J mice lacking melanocortin-3 or -4 receptors. Endocrinology 2006; 147(5): 2183-96.
[http://dx.doi.org/10.1210/en.2005-1209] [PMID: 16469808]

[103] Nahon JL, Presse F, Bittencourt JC, Sawchenko PE, Vale W. The rat melanin-concentrating hormone messenger ribonucleic acid encodes multiple putative neuropeptides coexpressed in the dorsolateral hypothalamus. Endocrinology 1989; 125(4): 2056-65.
[http://dx.doi.org/10.1210/endo-125-4-2056] [PMID: 2477226]

[104] Gomori A, Ishihara A, Ito M, *et al.* Chronic intracerebroventricular infusion of MCH causes obesity in mice. Am J Physiol Endocrinol Metab 2003; 284(3): E583-8.
[http://dx.doi.org/10.1152/ajpendo.00350.2002] [PMID: 12453827]

[105] Sailer AW, Sano H, Zeng Z, *et al.* Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. Proc Natl Acad Sci USA 2001; 98(13): 7564-9.
[http://dx.doi.org/10.1073/pnas.121170598] [PMID: 11404457]

[106] Marsh DJ, Weingarth DT, Novi DE, *et al.* Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc Natl Acad Sci USA 2002; 99(5): 3240-5.
[http://dx.doi.org/10.1073/pnas.052706899] [PMID: 11867747]

[107] Peyron C, Tighe DK, van den Pol AN, *et al.* Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18(23): 9996-10015.
[http://dx.doi.org/10.1523/JNEUROSCI.18-23-09996.1998] [PMID: 9822755]

[108] Sakurai T, *et al.* Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92, 1 page following 696 1998.
[http://dx.doi.org/10.1016/S0092-8674(00)80949-6]

[109] Yamanaka A, Sakurai T, Katsumoto T, Masashi Yanagisawa , Goto K. Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Res 1999; 849(1-2): 248-52.
[http://dx.doi.org/10.1016/S0006-8993(99)01905-8] [PMID: 10592311]

[110] Yamanaka A, Kunii K, Nambu T, *et al.* Orexin-induced food intake involves neuropeptide Y pathway. Brain Res 2000; 859(2): 404-9.
[http://dx.doi.org/10.1016/S0006-8993(00)02043-6] [PMID: 10719096]

[111] Sartin JL, Dyer C, Matteri R, *et al.* Effect of intracerebroventricular orexin-B on food intake in sheep. J Anim Sci 2001; 79(6): 1573-7.
[http://dx.doi.org/10.2527/2001.7961573x] [PMID: 11424696]

[112] Furuse M, Ando R, Bungo T, Shimojo M, Masuda Y, Masuda Y. Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. Br Poult Sci 1999; 40(5): 698-700.
[http://dx.doi.org/10.1080/00071669987115] [PMID: 10670685]

[113] Lassiter K, Greene E, Piekarski A, *et al.* Orexin system is expressed in avian muscle cells and regulates mitochondrial dynamics. Am J Physiol Regul Integr Comp Physiol 2015; 308(3): R173-87.
[http://dx.doi.org/10.1152/ajpregu.00394.2014] [PMID: 25502749]

[114] Kyrkouli SE, Glenn Stanley B, Leibowitz SF. Galanin: Stimulation of feeding induced by medial hypothalamic injection of this novel peptide. Eur J Pharmacol 1986; 122(1): 159-60.
[http://dx.doi.org/10.1016/0014-2999(86)90175-5] [PMID: 2420618]

[115] Tempel DL, Leibowitz KJ, Leibowitz SF. Effects of PVN galanin on macronutrient selection. Peptides 1988; 9(2): 309-14.
[http://dx.doi.org/10.1016/0196-9781(88)90265-3] [PMID: 2453854]

[116] Lang R, Gundlach A, Kofler B. The galanin peptide family: Receptor pharmacology, pleiotropic biological actions, and implications in health and disease. Pharmacol Ther 2007; 115(2): 177-207.
[http://dx.doi.org/10.1016/j.pharmthera.2007.05.009] [PMID: 17604107]

[117] Izdebska K, Ciosek J. Galanin influences on vasopressin and oxytocin release: *In vitro* studies. Neuropeptides 2010; 44(4): 341-8.
[http://dx.doi.org/10.1016/j.npep.2010.04.004] [PMID: 20466422]

[118] Lang R, Kofler B. The galanin peptide family in inflammation. Neuropeptides 2011; 45(1): 1-8.
[http://dx.doi.org/10.1016/j.npep.2010.10.005] [PMID: 21087790]

[119] Tachibana T, Mori M, Khan MSI, Ueda H, Sugahara K, Hiramatsu K. Central administration of galanin stimulates feeding behavior in chicks. Comp Biochem Physiol A Mol Integr Physiol 2008; 151(4): 637-40.
[http://dx.doi.org/10.1016/j.cbpa.2008.08.001] [PMID: 18725311]

[120] Ando R, Bungo T, Kawakami S-I, Shimojo M, Masuda Y, Furuse M. Intracerebroventricular injection of mammalian motilin, melanin-concentrating hormone or galanin does not stimulate food intake in neonatal chicks. Br Poult Sci 2000; 41(4): 508-11.
[http://dx.doi.org/10.1080/00071660050195037] [PMID: 11128394]

[121] Ohtaki T, Kumano S, Ishibashi Y, *et al.* Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. J Biol Chem 1999; 274(52): 37041-5.
[http://dx.doi.org/10.1074/jbc.274.52.37041] [PMID: 10601261]

[122] Cunningham MJ, Shahab M, Grove KL, *et al.* Galanin-like peptide as a possible link between metabolism and reproduction in the macaque. J Clin Endocrinol Metab 2004; 89(4): 1760-6.
[http://dx.doi.org/10.1210/jc.2003-031628] [PMID: 15070942]

[123] Matsumoto Y, Watanabe T, Adachi Y, *et al.* Galanin-like peptide stimulates food intake in the rat. Neurosci Lett 2002; 322(1): 67-9.
[http://dx.doi.org/10.1016/S0304-3940(01)02515-0] [PMID: 11958845]

[124] Lawrence CB, Baudoin FMH, Luckman SM. Centrally administered galanin-like peptide modifies food intake in the rat: a comparison with galanin. J Neuroendocrinol 2002; 14(11): 853-60.
[http://dx.doi.org/10.1046/j.1365-2826.2002.00846.x] [PMID: 12421338]

[125] Kuramochi M, Onaka T, Kohno D, Kato S, Yada T. Galanin-like peptide stimulates food intake *via* activation of neuropeptide Y neurons in the hypothalamic dorsomedial nucleus of the rat. Endocrinology 2006; 147(4): 1744-52.
[http://dx.doi.org/10.1210/en.2005-0907] [PMID: 16410310]

[126] Hansen KR, Krasnow SM, Nolan MA, *et al.* Activation of the sympathetic nervous system by galanin-like peptide--a possible link between leptin and metabolism. Endocrinology 2003; 144(11): 4709-17.
[http://dx.doi.org/10.1210/en.2003-0748] [PMID: 12960003]

[127] Kageyama H, Kita T, Toshinai K, *et al.* Galanin-like peptide promotes feeding behaviour *via* activation of orexinergic neurones in the rat lateral hypothalamus. J Neuroendocrinol 2006; 18(1): 33-41.
[http://dx.doi.org/10.1111/j.1365-2826.2005.01382.x] [PMID: 16451218]

[128] Gardiner JV, Beale KE, Roy D, *et al.* Cerebellin1 is a novel orexigenic peptide. Diabetes Obes Metab 2010; 12(10): 883-90.
[http://dx.doi.org/10.1111/j.1463-1326.2010.01247.x] [PMID: 20920041]

[129] Reiner A, Yang M, Cagle MC, Honig MG. Localization of cerebellin-2 in late embryonic chicken brain: Implications for a role in synapse formation and for brain evolution. J Comp Neurol 2011; 519(11): 2225-51.
[http://dx.doi.org/10.1002/cne.22626] [PMID: 21456003]

[130] Nauck MA, Niedereichholz U, Ettler R, *et al.* Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 1997; 273(5): E981-8.
[PMID: 9374685]

[131] Tang-Christensen M, Vrang N, Larsen P J. Larsen, Glucagon-like peptide containing pathways in the regulation of feeding behaviour. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2001; 25(Suppl 5): S42-47.

[132] Guan X, Shi X, Li X, *et al.* GLP-2 receptor in POMC neurons suppresses feeding behavior and gastric motility. Am J Physiol Endocrinol Metab 2012; 303(7): E853-64.
[http://dx.doi.org/10.1152/ajpendo.00245.2012] [PMID: 22829581]

[133] Furuse M, Bungo T, Shimojo M, *et al.* Effects of various N-terminal fragments of glucagon-like peptide-1(7–36) on food intake in the neonatal chick. Brain Res 1998; 807(1-2): 214-7.
[http://dx.doi.org/10.1016/S0006-8993(98)00798-7] [PMID: 9757042]

[134] Honda K, *et al.* Intracerebroventricular administration of chicken glucagon-like peptide-2 potently suppresses food intake in chicks. Animal science journal = Nihon chikusan Gakkaiho 2015; 86: 312-8.

[135] Khan MSI, Nakano Y, Tachibana T, Ueda H. Nitric oxide synthase inhibitor attenuates the anorexigenic effect of corticotropin-releasing hormone in neonatal chicks. Comp Biochem Physiol A Mol Integr Physiol 2008; 149(3): 325-9.
[http://dx.doi.org/10.1016/j.cbpa.2008.01.011] [PMID: 18280762]

[136] Beck B, Stricker-Krongrad A, Richy S, Burlet C. Evidence that hypothalamic neurotensin signals leptin effects on feeding behavior in normal and fat-preferring rats. Biochem Biophys Res Commun 1998; 252(3): 634-8.
[http://dx.doi.org/10.1006/bbrc.1998.9712] [PMID: 9837758]

[137] Masuda K, Tachibana T, Ukena K. Effects of neurotensin and LANT-6 on food intake in chicks. American Journal of Life Sciences 2015; 3(3): 17-23.
[http://dx.doi.org/10.11648/j.ajls.s.2015030302.14]

[138] Oh-I S, Shimizu H, Satoh T, *et al.* Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 2006; 443(7112): 709-12.

[http://dx.doi.org/10.1038/nature05162] [PMID: 17036007]

[139] Maejima Y, Sedbazar U, Suyama S, *et al.* Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. Cell Metab 2009; 10(5): 355-65.
[http://dx.doi.org/10.1016/j.cmet.2009.09.002] [PMID: 19883614]

[140] Shousha DKS, Naso T. Effect of central and peripheral nesfatin-1 on food intake in Japanese quail. AASCIT Journal of Biology 2015; 1: 1-9.

[141] Lawrence CB, Ellacott KLJ, Luckman SM. PRL-releasing peptide reduces food intake and may mediate satiety signaling. Endocrinology 2002; 143(2): 360-7.
[http://dx.doi.org/10.1210/endo.143.2.8609] [PMID: 11796487]

[142] Wang G, Tachibana T, Gilbert ER, Cline MA. Exogenous prolactin-releasing peptide's orexigenic effect is associated with hypothalamic neuropeptide Y in chicks. Neuropeptides 2015; 54: 79-83.
[http://dx.doi.org/10.1016/j.npep.2015.08.004] [PMID: 26349952]

[143] Ukena K. Avian and murine neurosecretory protein GL participates in the regulation of feeding and energy metabolism. Gen Comp Endocrinol 2018; 260: 164-70.
[http://dx.doi.org/10.1016/j.ygcen.2017.09.019] [PMID: 28951261]

[144] Ukena K, Iwakoshi-Ukena E, Taniuchi S, *et al.* Identification of a cDNA encoding a novel small secretory protein, neurosecretory protein GL, in the chicken hypothalamic infundibulum. Biochem Biophys Res Commun 2014; 446(1): 298-303.
[http://dx.doi.org/10.1016/j.bbrc.2014.02.090] [PMID: 24582750]

[145] Shikano K, Kato M, Iwakoshi-Ukena E, *et al.* Effects of chronic intracerebroventricular infusion of neurosecretory protein GL on body mass and food and water intake in chicks. Gen Comp Endocrinol 2018; 256: 37-42.
[http://dx.doi.org/10.1016/j.ygcen.2017.05.016] [PMID: 28554734]

[146] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 2013; 30(12): 2725-9.
[http://dx.doi.org/10.1093/molbev/mst197] [PMID: 24132122]

[147] Matsuura D, Shikano K, Saito T, *et al.* Neurosecretory Protein GL, a Hypothalamic Small Secretory Protein, Participates in Energy Homeostasis in Male Mice. Endocrinology 2017; 158(5): 1120-9.
[http://dx.doi.org/10.1210/en.2017-00064] [PMID: 28323972]

[148] Iwakoshi-Ukena E, Shikano K, Kondo K, *et al.* Neurosecretory protein GL stimulates food intake, de novo lipogenesis, and onset of obesity. eLife 2017; 6: e28527.
[http://dx.doi.org/10.7554/eLife.28527] [PMID: 28799896]

[149] Shikano K, Bessho Y, Kato M, *et al.* Localization and function of neurosecretory protein GM, a novel small secretory protein, in the chicken hypothalamus. Sci Rep 2018; 8(1): 704.
[http://dx.doi.org/10.1038/s41598-017-18822-9] [PMID: 29335496]

[150] Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol 2012; 13(4): 251-62.
[http://dx.doi.org/10.1038/nrm3311] [PMID: 22436748]

[151] Andersson U, Filipsson K, Abbott CR, *et al.* AMP-activated protein kinase plays a role in the control of food intake. J Biol Chem 2004; 279(13): 12005-8.
[http://dx.doi.org/10.1074/jbc.C300557200] [PMID: 14742438]

[152] Minokoshi Y, Alquier T, Furukawa N, *et al.* AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 2004; 428(6982): 569-74.
[http://dx.doi.org/10.1038/nature02440] [PMID: 15058305]

[153] Claret M, Smith MA, Batterham RL, *et al.* AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J Clin Invest 2007; 117(8): 2325-36.
[http://dx.doi.org/10.1172/JCI31516] [PMID: 17671657]

[154] Proszkowiec-Weglarz M, Richards MP, Ramachandran R, McMurtry JP. Characterization of the AMP-activated protein kinase pathway in chickens. Comp Biochem Physiol B Biochem Mol Biol 2006; 143(1): 92-106.
[http://dx.doi.org/10.1016/j.cbpb.2005.10.009] [PMID: 16343965]

[155] Liu L, Zhu L. Effect of 24 h Fasting on Gene Expression of AMPK, Appetite Regulation Peptides and Lipometabolism Related Factors in the Hypothalamus of Broiler Chicks. Asian-Australas J Anim Sci 2012; 25(9): 1300-8.
[http://dx.doi.org/10.5713/ajas.2012.12153] [PMID: 25049694]

[156] Xu P, Siegel PB, Denbow DM. AICAR and Compound C regulate food intake independently of AMP-activated protein kinase in lines of chickens selected for high or low body weight. Comp Biochem Physiol A Mol Integr Physiol 2011; 159(4): 401-12.
[http://dx.doi.org/10.1016/j.cbpa.2011.04.004] [PMID: 21530673]

[157] Piekarski A, Nagarajan G, Ishola P, *et al.* AMP-Activated Protein Kinase Mediates the Effect of Leptin on Avian Autophagy in a Tissue-Specific Manner. Front Physiol 2018; 9: 541.
[http://dx.doi.org/10.3389/fphys.2018.00541] [PMID: 29867578]

[158] Obici S, Feng Z, Arduini A, Conti R, Rossetti L. Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. Nat Med 2003; 9(6): 756-61.
[http://dx.doi.org/10.1038/nm873] [PMID: 12754501]

[159] Spector R. Fatty acid transport through the blood-brain barrier. J Neurochem 1988; 50(2): 639-43.
[http://dx.doi.org/10.1111/j.1471-4159.1988.tb02958.x] [PMID: 3335863]

[160] Le Foll C, Irani BG, Magnan C, Dunn-Meynell AA, Levin BE. Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing. Am J Physiol Regul Integr Comp Physiol 2009; 297(3): R655-64.
[http://dx.doi.org/10.1152/ajpregu.00223.2009] [PMID: 19535676]

[161] Wang R, Cruciani-Guglielmacci C, Migrenne S, Magnan C, Cotero VE, Routh VH. Effects of oleic acid on distinct populations of neurons in the hypothalamic arcuate nucleus are dependent on extracellular glucose levels. J Neurophysiol 2006; 95(3): 1491-8.
[http://dx.doi.org/10.1152/jn.00697.2005] [PMID: 16306178]

[162] Jo YH, Su Y, Gutierrez-Juarez R, Chua S Jr. Oleic acid directly regulates POMC neuron excitability in the hypothalamus. J Neurophysiol 2009; 101(5): 2305-16.
[http://dx.doi.org/10.1152/jn.91294.2008] [PMID: 19261705]

[163] Schwinkendorf DR, Tsatsos NG, Gosnell BA, Mashek DG. Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism. Int J Obes 2011; 35(3): 336-44.
[http://dx.doi.org/10.1038/ijo.2010.159] [PMID: 20714328]

[164] Obici S, Feng Z, Morgan K, Stein D, Karkanias G, Rossetti L. Central administration of oleic acid inhibits glucose production and food intake. Diabetes 2002; 51(2): 271-5.
[http://dx.doi.org/10.2337/diabetes.51.2.271] [PMID: 11812732]

[165] Lam TKT, Pocai A, Gutierrez-Juarez R, *et al.* Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. Nat Med 2005; 11(3): 320-7.
[http://dx.doi.org/10.1038/nm1201] [PMID: 15735652]

[166] Le Foll C, Dunn-Meynell A, Musatov S, Magnan C, Levin BE. FAT/CD36: a major regulator of neuronal fatty acid sensing and energy homeostasis in rats and mice. Diabetes 2013; 62(8): 2709-16.
[http://dx.doi.org/10.2337/db12-1689] [PMID: 23557700]

[167] Le Foll C, Dunn-Meynell AA, Levin BE. Role of FAT/CD36 in fatty acid sensing, energy, and glucose homeostasis regulation in DIO and DR rats. Am J Physiol Regul Integr Comp Physiol 2015; 308(3): R188-98.
[http://dx.doi.org/10.1152/ajpregu.00367.2014] [PMID: 25477422]

[168] Dridi S, Ververken C, Hillgartner FB, *et al.* FAS inhibitor cerulenin reduces food intake and melanocortin receptor gene expression without modulating the other (an)orexigenic neuropeptides in chickens. Am J Physiol Regul Integr Comp Physiol 2006; 291(1): R138-47.
[http://dx.doi.org/10.1152/ajpregu.00899.2005] [PMID: 16455759]

[169] Loftus TM, Jaworsky DE, Frehywot GL, *et al.* Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science 2000; 288(5475): 2379-81.
[http://dx.doi.org/10.1126/science.288.5475.2379] [PMID: 10875926]

[170] Kumar MV, Shimokawa T, Nagy TR, Lane MD. Differential effects of a centrally acting fatty acid synthase inhibitor in lean and obese mice. Proc Natl Acad Sci USA 2002; 99(4): 1921-5.
[http://dx.doi.org/10.1073/pnas.042683699] [PMID: 11854492]

[171] Gao S, Lane MD. Effect of the anorectic fatty acid synthase inhibitor C75 on neuronal activity in the hypothalamus and brainstem. Proc Natl Acad Sci USA 2003; 100(10): 5628-33.
[http://dx.doi.org/10.1073/pnas.1031698100] [PMID: 12724522]

[172] Shimokawa T, Kumar MV, Lane MD. Effect of a fatty acid synthase inhibitor on food intake and expression of hypothalamic neuropeptides. Proc Natl Acad Sci USA 2002; 99(1): 66-71.
[http://dx.doi.org/10.1073/pnas.012606199] [PMID: 11756683]

[173] Hu Z, Cha SH, Chohnan S, Lane MD. Hypothalamic malonyl-CoA as a mediator of feeding behavior. Proc Natl Acad Sci USA 2003; 100(22): 12624-9.
[http://dx.doi.org/10.1073/pnas.1834402100] [PMID: 14532332]

[174] Wolfgang MJ, Cha SH, Sidhaye A, *et al.* Regulation of hypothalamic malonyl-CoA by central glucose and leptin. Proc Natl Acad Sci USA 2007; 104(49): 19285-90.
[http://dx.doi.org/10.1073/pnas.0709778104] [PMID: 18032600]

[175] López M, Lage R, Saha AK, *et al.* Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. Cell Metab 2008; 7(5): 389-99.
[http://dx.doi.org/10.1016/j.cmet.2008.03.006] [PMID: 18460330]

[176] Gao S, Keung W, Serra D, *et al.* Malonyl-CoA mediates leptin hypothalamic control of feeding independent of inhibition of CPT-1a. Am J Physiol Regul Integr Comp Physiol 2011; 301(1): R209-17.
[http://dx.doi.org/10.1152/ajpregu.00092.2011] [PMID: 21508288]

[177] Gao S, Kinzig KP, Aja S, *et al.* Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. Proc Natl Acad Sci USA 2007; 104(44): 17358-63.
[http://dx.doi.org/10.1073/pnas.0708385104] [PMID: 17956983]

[178] Price NT, van der Leij FR, Jackson VN, *et al.* A novel brain-expressed protein related to carnitine palmitoyltransferase I. Genomics 2002; 80(4): 433-42.
[http://dx.doi.org/10.1006/geno.2002.6845] [PMID: 12376098]

[179] Sierra AY, Gratacós E, Carrasco P, *et al.* CPT1c is localized in endoplasmic reticulum of neurons and has carnitine palmitoyltransferase activity. J Biol Chem 2008; 283(11): 6878-85.
[http://dx.doi.org/10.1074/jbc.M707965200] [PMID: 18192268]

[180] Wolfgang MJ, Kurama T, Dai Y, *et al.* The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. Proc Natl Acad Sci USA 2006; 103(19): 7282-7.
[http://dx.doi.org/10.1073/pnas.0602205103] [PMID: 16651524]

[181] Gao S, Zhu G, Gao X, *et al.* Important roles of brain-specific carnitine palmitoyltransferase and ceramide metabolism in leptin hypothalamic control of feeding. Proc Natl Acad Sci USA 2011; 108(23): 9691-6.
[http://dx.doi.org/10.1073/pnas.1103267108] [PMID: 21593415]

[182] Carrasco P, Sahún I, McDonald J, *et al.* Ceramide levels regulated by carnitine palmitoyltransferase 1C control dendritic spine maturation and cognition. J Biol Chem 2012; 287(25): 21224-32.
[http://dx.doi.org/10.1074/jbc.M111.337493] [PMID: 22539351]

[183] Wang H, Eckel RH. What are lipoproteins doing in the brain? Trends Endocrinol Metab 2014; 25(1): 8-14.
[http://dx.doi.org/10.1016/j.tem.2013.10.003] [PMID: 24189266]

[184] Wang H, Eckel RH. Lipoprotein lipase in the brain and nervous system. Annu Rev Nutr 2012; 32(1): 147-60.
[http://dx.doi.org/10.1146/annurev-nutr-071811-150703] [PMID: 22540257]

[185] Laperrousaz E, Moullé VS, Denis RG, *et al.* Lipoprotein lipase in hypothalamus is a key regulator of body weight gain and glucose homeostasis in mice. Diabetologia 2017; 60(7): 1314-24.
[http://dx.doi.org/10.1007/s00125-017-4282-7] [PMID: 28456865]

[186] Picard A, Rouch C, Kassis N, *et al.* Hippocampal lipoprotein lipase regulates energy balance in rodents. Mol Metab 2014; 3(2): 167-76.
[http://dx.doi.org/10.1016/j.molmet.2013.11.002] [PMID: 24634821]

[187] Mayer J. Glucostatic mechanism of regulation of food intake. N Engl J Med 1953; 249(1): 13-6.
[http://dx.doi.org/10.1056/NEJM195307022490104] [PMID: 13063674]

[188] Louis-Sylvestre J, Le Magnen J. A fall in blood glucose level precedes meal onset in free-feeding rats. Neurosci Biobehav Rev 1980; 4 (Suppl. 1): 13-5.
[http://dx.doi.org/10.1016/0149-7634(80)90041-X] [PMID: 6765504]

[189] Campfield LA, Smith FJ. Functional coupling between transient declines in blood glucose and feeding behavior: Temporal relationships. Brain Res Bull 1986; 17(3): 427-33.
[http://dx.doi.org/10.1016/0361-9230(86)90250-9] [PMID: 3768746]

[190] Campfield LA, Smith FJ. Transient declines in blood glucose signal meal initiation. Int J Obes 1990; 14 (Suppl. 3): 15-31.
[PMID: 2086508]

[191] Mayer J. Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. Ann N Y Acad Sci 1955; 63(1): 15-43.
[http://dx.doi.org/10.1111/j.1749-6632.1955.tb36543.x] [PMID: 13249313]

[192] Bray GA. Static theories in a dynamic world: a glucodynamic theory of food intake. Obes Res 1996; 4(5): 489-92.
[http://dx.doi.org/10.1002/j.1550-8528.1996.tb00259.x] [PMID: 8885215]

[193] Silver IA, Erecińska M. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. J Neurosci 1994; 14(8): 5068-76.
[http://dx.doi.org/10.1523/JNEUROSCI.14-08-05068.1994] [PMID: 8046468]

[194] Anand BK, Chhina GS, Sharma KN, Dua S, Singh B. Activity of single neurons in the hypothalamic feeding centers: effect of glucose. Am J Physiol 1964; 207(5): 1146-54.
[http://dx.doi.org/10.1152/ajplegacy.1964.207.5.1146] [PMID: 14237464]

[195] Oomura Y, Kimura K, Ooyama H, Maeno T, Iki M, Kuniyoshi M. Reciprocal Activities of the Ventromedial and Lateral Hypothalamic Areas of Cats. Science 1964; 143(3605): 484-5.
[http://dx.doi.org/10.1126/science.143.3605.484] [PMID: 14080319]

[196] Wang R, Liu X, Hentges ST, *et al.* The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. Diabetes 2004; 53(8): 1959-65.
[http://dx.doi.org/10.2337/diabetes.53.8.1959] [PMID: 15277373]

[197] Routh VH, Hao L, Santiago AM, Sheng Z, Zhou C. Hypothalamic glucose sensing: making ends meet. Front Syst Neurosci 2014; 8: 236.
[http://dx.doi.org/10.3389/fnsys.2014.00236] [PMID: 25540613]

[198] Dunn-Meynell AA, Routh VH, Kang L, Gaspers L, Levin BE. Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. Diabetes 2002; 51(7):

2056-65.
[http://dx.doi.org/10.2337/diabetes.51.7.2056] [PMID: 12086933]

[199] Kang L, Dunn-Meynell AA, Routh VH, *et al.* Glucokinase is a critical regulator of ventromedial hypothalamic neuronal glucosensing. Diabetes 2006; 55(2): 412-20.
[http://dx.doi.org/10.2337/diabetes.55.02.06.db05-1229] [PMID: 16443775]

[200] Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE. Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. Diabetes 2004; 53(3): 549-59.
[http://dx.doi.org/10.2337/diabetes.53.3.549] [PMID: 14988237]

[201] Murphy BA, Fioramonti X, Jochnowitz N, *et al.* Fasting enhances the response of arcuate neuropeptide Y-glucose-inhibited neurons to decreased extracellular glucose. Am J Physiol Cell Physiol 2009; 296(4): C746-56.
[http://dx.doi.org/10.1152/ajpcell.00641.2008] [PMID: 19211911]

[202] Joly-Amado A, Denis RGP, Castel J, *et al.* Hypothalamic AgRP-neurons control peripheral substrate utilization and nutrient partitioning. EMBO J 2012; 31(22): 4276-88.
[http://dx.doi.org/10.1038/emboj.2012.250] [PMID: 22990237]

[203] Williams RH, Alexopoulos H, Jensen LT, Fugger L, Burdakov D. Adaptive sugar sensors in hypothalamic feeding circuits. Proc Natl Acad Sci USA 2008; 105(33): 11975-80.
[http://dx.doi.org/10.1073/pnas.0802687105] [PMID: 18695235]

[204] Burdakov D, Gerasimenko O, Verkhratsky A. Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons *in situ.* J Neurosci 2005; 25(9): 2429-33.
[http://dx.doi.org/10.1523/JNEUROSCI.4925-04.2005] [PMID: 15745970]

[205] Shram NF, Netchiporouk LI, Martelet C, Jaffrezic-Renault N, Cespuglio R. Brain glucose. Neuroreport 1997; 8(5): 1109-12.
[http://dx.doi.org/10.1097/00001756-199703240-00009] [PMID: 9175094]

[206] García MA, Millán C, Balmaceda-Aguilera C, *et al.* Hypothalamic ependymal-glial cells express the glucose transporter GLUT2, a protein involved in glucose sensing. J Neurochem 2003; 86(3): 709-24.
[http://dx.doi.org/10.1046/j.1471-4159.2003.01892.x] [PMID: 12859684]

[207] Salgado M, Tarifeño-Saldivia E, Ordenes P, *et al.* Dynamic localization of glucokinase and its regulatory protein in hypothalamic tanycytes. PLoS One 2014; 9(4): e94035.
[http://dx.doi.org/10.1371/journal.pone.0094035] [PMID: 24739934]

[208] Elizondo-Vega R, Cortés-Campos C, Barahona MJ, *et al.* Inhibition of hypothalamic MCT1 expression increases food intake and alters orexigenic and anorexigenic neuropeptide expression. Sci Rep 2016; 6(1): 33606.
[http://dx.doi.org/10.1038/srep33606] [PMID: 27677351]

[209] Uranga RM, Millán C, Barahona MJ, *et al.* Adenovirus-mediated suppression of hypothalamic glucokinase affects feeding behavior. Sci Rep 2017; 7(1): 3697.
[http://dx.doi.org/10.1038/s41598-017-03928-x] [PMID: 28623340]

[210] Barahona MJ, Llanos P, Recabal A, *et al.* Glial hypothalamic inhibition of GLUT2 expression alters satiety, impacting eating behavior. Glia 2018; 66(3): 592-605.
[http://dx.doi.org/10.1002/glia.23267] [PMID: 29178321]

[211] Benford H, Bolborea M, Pollatzek E, *et al.* A sweet taste receptor-dependent mechanism of glucosensing in hypothalamic tanycytes. Glia 2017; 65(5): 773-89.
[http://dx.doi.org/10.1002/glia.23125] [PMID: 28205335]

[212] Mellinkoff SM, Frankland M, Boyle D, Greipel M. Relationship between serum amino acid concentration and fluctuations in appetite. J Appl Physiol 1956; 8(5): 535-8.
[http://dx.doi.org/10.1152/jappl.1956.8.5.535] [PMID: 13295170]

[213] Anderson GH, Tecimer SN, Shah D, Zafar TA. Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men. J Nutr 2004; 134(11): 3011-5. [http://dx.doi.org/10.1093/jn/134.11.3011] [PMID: 15514267]

[214] Li ETS, Anderson GH. Meal composition influences subsequent food selection in the young rat. Physiol Behav 1982; 29(5): 779-83. [http://dx.doi.org/10.1016/0031-9384(82)90324-9] [PMID: 7156215]

[215] Anderson GH, Li ET. Protein and amino acids in the regulation of quantitative and qualitative aspects of food intake. Int J Obes 1987; 11 (Suppl. 3): 97-108. [PMID: 3326851]

[216] Foster GD, Wyatt HR, Hill JO, *et al.* A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003; 348(21): 2082-90. [http://dx.doi.org/10.1056/NEJMoa022207] [PMID: 12761365]

[217] Gous RM, Griessel M, Morris TR. Effect of dietary energy concentration on the response of laying hens to amino acids. Br Poult Sci 1987; 28(3): 427-36. [http://dx.doi.org/10.1080/00071668708416977] [PMID: 3119163]

[218] Burnham D, Emmans GC, Gous RM. Isoleucine requirements of the chicken: The effect of excess leucine and valine on the response to isoleucine. Br Poult Sci 1992; 33(1): 71-87. [http://dx.doi.org/10.1080/00071669208417445] [PMID: 1571809]

[219] Henry Y. Dietary factors involved in feed intake regulation in growing pigs: A review. Livest Prod Sci 1985; 12(4): 339-54. [http://dx.doi.org/10.1016/0301-6226(85)90133-2]

[220] Allen MS. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J Dairy Sci 2000; 83(7): 1598-624. [http://dx.doi.org/10.3168/jds.S0022-0302(00)75030-2] [PMID: 10908065]

[221] Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, *et al.* Dose-dependent satiating effect of whey relative to casein or soy. Physiol Behav 2009; 96(4-5): 675-82. [http://dx.doi.org/10.1016/j.physbeh.2009.01.004] [PMID: 19385022]

[222] Anderson GH, Li ET, Anthony SP, Ng LT, Bialik R. Dissociation between plasma and brain amino acid profiles and short-term food intake in the rat. Am J Physiol 1994; 266(5 Pt 2): R1675-86. [PMID: 8203650]

[223] Wang Y, Chandra R, Samsa LA, *et al.* Amino acids stimulate cholecystokinin release through the $Ca^{2+}$-sensing receptor. Am J Physiol Gastrointest Liver Physiol 2011; 300(4): G528-37. [http://dx.doi.org/10.1152/ajpgi.00387.2010] [PMID: 21183662]

[224] Reidelberger RD. Cholecystokinin and control of food intake. J Nutr 1994; 124(8) (Suppl. 8): 1327S-33S. [http://dx.doi.org/10.1093/jn/124.suppl_8.1327S] [PMID: 8064379]

[225] Crovetti R, Porrini M, Santangelo A, Testolin G. The influence of thermic effect of food on satiety. Eur J Clin Nutr 1998; 52(7): 482-8. [http://dx.doi.org/10.1038/sj.ejcn.1600578] [PMID: 9683329]

[226] Morens C, Bos C, Pueyo ME, *et al.* Increasing habitual protein intake accentuates differences in postprandial dietary nitrogen utilization between protein sources in humans. J Nutr 2003; 133(9): 2733-40. [http://dx.doi.org/10.1093/jn/133.9.2733] [PMID: 12949358]

[227] Gietzen DW, Magrum LJ. Molecular mechanisms in the brain involved in the anorexia of branched-chain amino acid deficiency. J Nutr 2001; 131(3): 851S-5S. [http://dx.doi.org/10.1093/jn/131.3.851S] [PMID: 11238773]

[228] Zaragozá R. Transport of Amino Acids Across the Blood-Brain Barrier. Front Physiol 2020; 11: 973.

[http://dx.doi.org/10.3389/fphys.2020.00973] [PMID: 33071801]

[229] Tackman JM, Tews JK, Harper AE. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. J Nutr 1990; 120(5): 521-33.
[http://dx.doi.org/10.1093/jn/120.5.521] [PMID: 1692873]

[230] Gietzen DW, Erecius LF, Rogers QR. Neurochemical changes after imbalanced diets suggest a brain circuit mediating anorectic responses to amino acid deficiency in rats. J Nutr 1998; 128(4): 771-81.
[http://dx.doi.org/10.1093/jn/128.4.771] [PMID: 9521642]

[231] Gietzen DW. Neural mechanisms in the responses to amino acid deficiency. J Nutr 1993; 123(4): 610-25.
[http://dx.doi.org/10.1093/jn/123.4.610] [PMID: 8463862]

[232] Tews JK, Repa JJ, Harper AE. Norleucine: A branched-chain amino acid analog affecting feeding behavior of rats. Pharmacol Biochem Behav 1990; 35(4): 911-21.
[http://dx.doi.org/10.1016/0091-3057(90)90379-V] [PMID: 2345765]

[233] Leung PM, Rogers QR. Importance of prepyriform cortex in food-intake response of rats to amino acids. Am J Physiol 1971; 221(3): 929-35.
[http://dx.doi.org/10.1152/ajplegacy.1971.221.3.929] [PMID: 5570352]

[234] Magrum LJ, Hickman MA, Gietzen DW. Increased intracellular calcium in rat anterior piriform cortex in response to threonine after threonine deprivation. J Neurophysiol 1999; 81(3): 1147-9.
[http://dx.doi.org/10.1152/jn.1999.81.3.1147] [PMID: 10085341]

[235] Beverly JL III, Gietzen DW, Rogers QR. Protein synthesis in the prepyriform cortex: effects on intake of an amino acid-imbalanced diet by Sprague-Dawley rats. J Nutr 1991; 121(5): 754-61.
[http://dx.doi.org/10.1093/jn/121.5.754] [PMID: 2019884]

[236] Currie PJ, Chang N, Luo S, Anderson GH. Microdialysis as a tool to measure dietary and regional effects on the complete profile of extracellular amino acids in the hypothalamus of rats. Life Sci 1995; 57(21): 1911-23.
[http://dx.doi.org/10.1016/0024-3205(95)02178-L] [PMID: 7475941]

[237] Bjordal M, Arquier N, Kniazeff J, Pin JP, Léopold P. Sensing of amino acids in a dopaminergic circuitry promotes rejection of an incomplete diet in Drosophila. Cell 2014; 156(3): 510-21.
[http://dx.doi.org/10.1016/j.cell.2013.12.024] [PMID: 24485457]

[238] Zhang P, McGrath BC, Reinert J, *et al.* The GCN2 eIF2alpha kinase is required for adaptation to amino acid deprivation in mice. Mol Cell Biol 2002; 22(19): 6681-8.
[http://dx.doi.org/10.1128/MCB.22.19.6681-6688.2002] [PMID: 12215525]

[239] Maurin AC, Jousse C, Averous J, *et al.* The GCN2 kinase biases feeding behavior to maintain amino acid homeostasis in omnivores. Cell Metab 2005; 1(4): 273-7.
[http://dx.doi.org/10.1016/j.cmet.2005.03.004] [PMID: 16054071]

[240] Dong J, Qiu H, Garcia-Barrio M, Anderson J, Hinnebusch AG. Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain. Mol Cell 2000; 6(2): 269-79.
[http://dx.doi.org/10.1016/S1097-2765(00)00028-9] [PMID: 10983975]

[241] Panksepp J, Booth DA. Decreased feeding after injections of amino-acids into the hypothalamus. Nature 1971; 233(5318): 341-2.
[http://dx.doi.org/10.1038/233341a0] [PMID: 4940431]

[242] Blouet C, Jo YH, Li X, Schwartz GJ. Mediobasal hypothalamic leucine sensing regulates food intake through activation of a hypothalamus-brainstem circuit. J Neurosci 2009; 29(26): 8302-11.
[http://dx.doi.org/10.1523/JNEUROSCI.1668-09.2009] [PMID: 19571121]

[243] Laeger T, Reed SD, Henagan TM, *et al.* Leucine acts in the brain to suppress food intake but does not function as a physiological signal of low dietary protein. Am J Physiol Regul Integr Comp Physiol 2014; 307(3): R310-20.

[http://dx.doi.org/10.1152/ajpregu.00116.2014] [PMID: 24898843]

[244] Gloaguen M, Le Floc'h N, Corrent E, Primot Y, van Milgen J. Providing a diet deficient in valine but with excess leucine results in a rapid decrease in feed intake and modifies the postprandial plasma amino acid and α-keto acid concentrations in pigs1. J Anim Sci 2012; 90(9): 3135-42.
[http://dx.doi.org/10.2527/jas.2011-4956] [PMID: 22585822]

[245] Cota D, Proulx K, Smith KAB, *et al.* Hypothalamic mTOR signaling regulates food intake. Science 2006; 312(5775): 927-30.
[http://dx.doi.org/10.1126/science.1124147] [PMID: 16690869]

[246] Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. Cell 2017; 169(2): 361-71.
[http://dx.doi.org/10.1016/j.cell.2017.03.035] [PMID: 28388417]

[247] Wu Q, Zhang Y, Xu J, Shen P. Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in Drosophila. Proc Natl Acad Sci USA 2005; 102(37): 13289-94.
[http://dx.doi.org/10.1073/pnas.0501914102] [PMID: 16150727]

[248] Morrison CD, Xi X, White CL, Ye J, Martin RJ. Amino acids inhibit *Agrp* gene expression via an mTOR-dependent mechanism. Am J Physiol Endocrinol Metab 2007; 293(1): E165-71.
[http://dx.doi.org/10.1152/ajpendo.00675.2006] [PMID: 17374702]

[249] Heeley N, Blouet C. Central Amino Acid Sensing in the Control of Feeding Behavior. Front Endocrinol (Lausanne) 2016; 7: 148.
[http://dx.doi.org/10.3389/fendo.2016.00148] [PMID: 27933033]

[250] Hill CM, Laeger T, Dehner M, *et al.* FGF21 Signals Protein Status to the Brain and Adaptively Regulates Food Choice and Metabolism. Cell Rep 2019; 27(10): 2934-2947.e3.
[http://dx.doi.org/10.1016/j.celrep.2019.05.022] [PMID: 31167139]

[251] Elise Rødland G, Tvegård T, Boye E, Grallert B. Crosstalk between the Tor and Gcn2 pathways in response to different stresses. Cell Cycle 2014; 13(3): 453-61.
[http://dx.doi.org/10.4161/cc.27270] [PMID: 24280780]

[252] Cherkasova VA, Hinnebusch AG. Translational control by TOR and TAP42 through dephosphorylation of eIF2α kinase GCN2. Genes Dev 2003; 17(7): 859-72.
[http://dx.doi.org/10.1101/gad.1069003] [PMID: 12654728]

[253] Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75(5): 843-54.
[http://dx.doi.org/10.1016/0092-8674(93)90529-Y] [PMID: 8252621]

[254] Gregory RI, Yan K, Amuthan G, *et al.* The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432(7014): 235-40.
[http://dx.doi.org/10.1038/nature03120] [PMID: 15531877]

[255] Lee Y, Ahn C, Han J, *et al.* The nuclear RNase III Drosha initiates microRNA processing. Nature 2003; 425(6956): 415-9.
[http://dx.doi.org/10.1038/nature01957] [PMID: 14508493]

[256] Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science 2004; 303(5654): 95-8.
[http://dx.doi.org/10.1126/science.1090599] [PMID: 14631048]

[257] Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 2005; 123(4): 631-40.
[http://dx.doi.org/10.1016/j.cell.2005.10.022] [PMID: 16271387]

[258] Chendrimada TP, Finn KJ, Ji X, *et al.* MicroRNA silencing through RISC recruitment of eIF6. Nature 2007; 447(7146): 823-8.
[http://dx.doi.org/10.1038/nature05841] [PMID: 17507929]

[259] Díaz NF, Cruz-Reséndiz MS, Flores-Herrera H, García-López G, Molina-Hernández A. MicroRNAs in central nervous system development. Rev Neurosci 2014; 25(5): 675-86.
[PMID: 24902008]

[260] Mercader JM, González JR, Lozano JJ, *et al.* Aberrant brain microRNA target and miRISC gene expression in the anx/anx anorexia mouse model. Gene 2012; 497(2): 181-90.
[http://dx.doi.org/10.1016/j.gene.2012.01.057] [PMID: 22310387]

[261] Schneeberger M, Altirriba J, García A, *et al.* Deletion of miRNA processing enzyme Dicer in POMC-expressing cells leads to pituitary dysfunction, neurodegeneration and development of obesity. Mol Metab 2013; 2(2): 74-85.
[http://dx.doi.org/10.1016/j.molmet.2012.10.001] [PMID: 24199146]

[262] Vinnikov IA, Hajdukiewicz K, Reymann J, *et al.* Hypothalamic miR-103 protects from hyperphagic obesity in mice. J Neurosci 2014; 34(32): 10659-74.
[http://dx.doi.org/10.1523/JNEUROSCI.4251-13.2014] [PMID: 25100599]

[263] Amar L, Benoit C, Beaumont G, *et al.* MicroRNA expression profiling of hypothalamic arcuate and paraventricular nuclei from single rats using Illumina sequencing technology. J Neurosci Methods 2012; 209(1): 134-43.
[http://dx.doi.org/10.1016/j.jneumeth.2012.05.033] [PMID: 22687940]

[264] Herzer S, Silahtaroglu A, Meister B. Locked nucleic acid-based *in situ* hybridisation reveals miR-7a as a hypothalamus-enriched microRNA with a distinct expression pattern. J Neuroendocrinol 2012; 24(12): 1492-504.
[http://dx.doi.org/10.1111/j.1365-2826.2012.02358.x] [PMID: 22775435]

[265] Crépin D, Benomar Y, Riffault L, Amine H, Gertler A, Taouis M. The over-expression of miR-200a in the hypothalamus of ob/ob mice is linked to leptin and insulin signaling impairment. Mol Cell Endocrinol 2014; 384(1-2): 1-11.
[http://dx.doi.org/10.1016/j.mce.2013.12.016] [PMID: 24394757]

[266] Sangiao-Alvarellos S, Pena-Bello L, Manfredi-Lozano M, Tena-Sempere M, Cordido F. Perturbation of hypothalamic microRNA expression patterns in male rats after metabolic distress: impact of obesity and conditions of negative energy balance. Endocrinology 2014; 155(5): 1838-50.
[http://dx.doi.org/10.1210/en.2013-1770] [PMID: 24517225]

[267] Derghal A, Djelloul M, Airault C, *et al.* Leptin is required for hypothalamic regulation of miRNAs targeting POMC 3â€²UTR. Front Cell Neurosci 2015; 9: 172.
[http://dx.doi.org/10.3389/fncel.2015.00172] [PMID: 25999818]

[268] Dubinsky AN, Dastidar SG, Hsu CL, *et al.* Let-7 coordinately suppresses components of the amino acid sensing pathway to repress mTORC1 and induce autophagy. Cell Metab 2014; 20(4): 626-38.
[http://dx.doi.org/10.1016/j.cmet.2014.09.001] [PMID: 25295787]

[269] Doherty J, Baehrecke EH. Life, death and autophagy. Nat Cell Biol 2018; 20(10): 1110-7.
[http://dx.doi.org/10.1038/s41556-018-0201-5] [PMID: 30224761]

[270] Mortimore GE, Hutson NJ, Surmacz CA. Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. Proc Natl Acad Sci USA 1983; 80(8): 2179-83.
[http://dx.doi.org/10.1073/pnas.80.8.2179] [PMID: 6340116]

[271] Mijaljica D, Prescott M, Devenish RJ. Microautophagy in mammalian cells: Revisiting a 40-year-old conundrum. Autophagy 2011; 7(7): 673-82.
[http://dx.doi.org/10.4161/auto.7.7.14733] [PMID: 21646866]

[272] Massey A, Kiffin R, Cuervo AM. Pathophysiology of chaperone-mediated autophagy. Int J Biochem Cell Biol 2004; 36(12): 2420-34.
[http://dx.doi.org/10.1016/j.biocel.2004.04.010] [PMID: 15325582]

[273] Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis

of mammalian Atg proteins. Autophagy 2010; 6(6): 764-76.
[http://dx.doi.org/10.4161/auto.6.6.12709] [PMID: 20639694]

[274] Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 2009; 5(8): 1180-5.
[http://dx.doi.org/10.4161/auto.5.8.10274] [PMID: 19855179]

[275] Hailey DW, Rambold AS, Satpute-Krishnan P, *et al.* Mitochondria supply membranes for autophagosome biogenesis during starvation. Cell 2010; 141(4): 656-67.
[http://dx.doi.org/10.1016/j.cell.2010.04.009] [PMID: 20478256]

[276] Takahashi Y, Meyerkord CL, Hori T, *et al.* Bif-1 regulates Atg9 trafficking by mediating the fission of Golgi membranes during autophagy. Autophagy 2011; 7(1): 61-73.
[http://dx.doi.org/10.4161/auto.7.1.14015] [PMID: 21068542]

[277] Hara T, Takamura A, Kishi C, *et al.* FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. J Cell Biol 2008; 181(3): 497-510.
[http://dx.doi.org/10.1083/jcb.200712064] [PMID: 18443221]

[278] Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal 2014; 20(3): 460-73.
[http://dx.doi.org/10.1089/ars.2013.5371] [PMID: 23725295]

[279] Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol Cell Biol 2001; 2(3): 211-6.
[http://dx.doi.org/10.1038/35056522] [PMID: 11265251]

[280] Weidberg H, Shvets E, Shpilka T, Shimron F, Shinder V, Elazar Z. LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. EMBO J 2010; 29(11): 1792-802.
[http://dx.doi.org/10.1038/emboj.2010.74] [PMID: 20418806]

[281] Liang C, Lee J, Inn KS, *et al.* Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. Nat Cell Biol 2008; 10(7): 776-87.
[http://dx.doi.org/10.1038/ncb1740] [PMID: 18552835]

[282] Fader CM, Sánchez DG, Mestre MB, Colombo MI. TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. Biochim Biophys Acta Mol Cell Res 2009; 1793(12): 1901-16.
[http://dx.doi.org/10.1016/j.bbamcr.2009.09.011] [PMID: 19781582]

[283] Itakura E, Kishi-Itakura C, Mizushima N. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell 2012; 151(6): 1256-69.
[http://dx.doi.org/10.1016/j.cell.2012.11.001] [PMID: 23217709]

[284] Singh R. Autophagy in the control of food intake. Adipocyte 2012; 1(2): 75-9.
[http://dx.doi.org/10.4161/adip.18966] [PMID: 23700515]

[285] Kaushik S, Rodriguez-Navarro JA, Arias E, *et al.* Autophagy in hypothalamic AgRP neurons regulates food intake and energy balance. Cell Metab 2011; 14(2): 173-83.
[http://dx.doi.org/10.1016/j.cmet.2011.06.008] [PMID: 21803288]

[286] Meng Q, Cai D. Defective hypothalamic autophagy directs the central pathogenesis of obesity *via* the IkappaB kinase beta (IKKbeta)/NF-kappaB pathway. J Biol Chem 2011; 286(37): 32324-32.
[http://dx.doi.org/10.1074/jbc.M111.254417] [PMID: 21784844]

[287] Xiao Y, Deng Y, Yuan F, *et al.* ATF4/ATG5 Signaling in Hypothalamic Proopiomelanocortin Neurons Regulates Fat Mass *via* Affecting Energy Expenditure. Diabetes 2017; 66(5): 1146-58.
[http://dx.doi.org/10.2337/db16-1546] [PMID: 28213613]

[288] Malhotra R, Warne JP, Salas E, Xu AW, Debnath J. Loss of Atg12, but not Atg5, in pro-opiomelanocortin neurons exacerbates diet-induced obesity. Autophagy 2015; 11(1): 145-54.
[PMID: 25585051]

[289] Schneeberger M, Dietrich MO, Sebastián D, *et al.* Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance. Cell 2013; 155(1): 172-87. [http://dx.doi.org/10.1016/j.cell.2013.09.003] [PMID: 24074867]

# CHAPTER 5

# Regulation of Food Intake: Peripheral Mechanisms

**Abstract:** In addition to central mechanisms, food intake is regulated by peripheral pathways, including gastric distension and the release of peripheral peptide signals that communicate with the hypothalamus to induce appetite and hunger or satiety and satiation. The present chapter summarizes the current knowledge related to peripheral peptides involved in the short- and long-term regulation of food intake.

**Keywords:** Long-term regulation of food intake, Peripheral peptides, Peripheral signaling, Short-term regulation of food intake.

## INTRODUCTION

The maintenance of the body weight set point (*i.e*. at a stable level) is a major determinant in keeping the higher animals survive [1]. As described in previous chapters, it is the result of a balance between energy intake and energy expenditure [2]. Animals take in energy through food and drink consumption and expend energy *via* the resting metabolic rate, the thermic effect of food, and physical activity. A dysregulation of this balance can lead to a change in body weight: increased BW when energy intake exceeds energy expenditure, or decreased BW when the energy expenditure surmounts the energy intake [3]. The energy (um)balance and its components continuously change over time, and they are regulated by complex physiological, metabolic, and molecular control systems [4 - 8]. As described in Chapter 4, there has been tremendous progress in identifying the important role of the CNS and the hypothalamus in the homeostatic regulation of energy balance. However, the central system is not working independently, but it rather interacts and crosstalk with the peripheral system. In fact, under steady-state conditions, ingested nutrients (energy intake) are digested, absorbed, metabolized, and stored. A neural regulator senses fuel (energy) availability in the internal milieu (nutrient sensing) and generates appropriate (satiety and adiposity) signals to the neural circuits controlling food intake and energy expenditure. The key components of the peripheral system are the gustatory system [9, 10], gastrointestinal tract [11, 12], pancreas [13], liver [14], muscle [15], and adipose tissue [16, 17]. All these components are in complex interplay and multidirectional communication with the brain *via* either

neural connections, hormones and/or metabolites. This peripheral regulation of food intake can be categorized in a short-term regulation, which determines the beginning and the end of a meal (hunger and satiation), and a long-term control *via* adiposity signals, which monitor energy storage and helps regulating the body energy depots [18]. The purpose of the present chapter is to provide an overview of peripheral pathways and summarize the current knowledge related to peripheral peptides involved in the short- and long-term regulation of food intake.

## 5.1. Short-term Regulation of Food Intake

Quartermain and coworkers [19] divided the short-term component of food intake into two phases: early pre-absorptive and delayed absorptive or digestive phases. The decision of meal initiation is mostly dictated by food availability, social conventions, and learned associations with physiological signals playing a relatively minor role [20]. However, data have suggested a role for the gastric peptides. The CNS uses inputs from mechanoreceptors in the stomach and the GI to determine how much food is enough. Shortly after food ingestion, the presence of nutrients in the stomach and proximal small intestine, as well as nutrients arriving *via* the portal vein, activate afferent signal that travel in vagal nerve fibers and control meal termination. Food ingestion releases gastrointestinal hormones and activates gastrointestinal motility, gastric, and pancreatico-biliary secretion and absorption. It has been estimated that the motor and secretory activities in the upper and mid-gut contribute to over 50% of the overall postprandial response [21]. The gut or pancreatic hormones, secreted from the mucosal enteroendocrine cells, act on vagal or other pathways (gut-brain interaction) to induce (*e.g*. ghrelin) or inhibit appetite and food intake (*e.g*. CCK, GLP-1, PYY, *etc*.).

### *5.1.1. Ghrelin*

Peripheral or central administration of ghrelin stimulated food intake at times when feeding would not normally occur and increased meal numbers without changing meal sizes [22], indicating that ghrelin regulates meal initiation [23]. In support of these data, the pattern of human plasma ghrelin is characterized by an increase before meals and a rapid decline after food consumption [24]. In rodents, circulating ghrelin rises during food deprivation and before the dark cycle, which is known as the main period for food consumption [25]. In avian species, however, ghrelin seemed to have the opposite effects compared to mammalian species [26]. This divergent action of ghrelin on food intake seems to be specie-specific.

Ghrelin is a 28-amino acid peptide, produced mainly in the oxyntic gland cells in the mucosa of the mammalian stomach [27] and avian proventriculus [28]. It has been reported that it is also expressed in other tissues such as the intestine, lung,

and brain [28, 29]. Ghrelin exerts its effect *via* growth hormone secretagogue receptor (GHS-R), which has been found to be expressed in many tissues, including the stomach innervating vagal afferent neurons in the nodose ganglion [30]. GHS-R is also expressed in hypothalamic NPY neurons [31]. Peripheral or central administration of ghrelin induces c-fos immunoreactivity in the hypothalamic ARC, PVN, and LHA [32]. Furthermore, ghrelin administration modulates the expression of hypothalamic NPY, AgRP, and orexin independently of the nutritional status [33 - 35], and antibodies and antagonists of NPY and AgRP abolish ghrelin-induced feeding behavior [34]. Ghrelin injection blocks leptin-induced feeding reduction, and ghrelin orexigenic effect is inhibited by central administration of Y1 receptor antagonist [36]. The peripheral and central effects of ghrelin on energy balance are likely to reflect complex interactions of NPY, AgRP, orexin, CRF, and other hypothalamic feeding-related neuropeptides [37].

### *5.1.2. CCK*

CCK, literary "bile-sack-move" from Greek word, was originally called pancreozymin because it is mainly synthesized and secreted by duodenal enteroendocrine cells where it plays a crucial role in the release of pancreatic exocrine enzymes, gallbladder contraction *via* the sphincter of Oddi and release of bile acids, and gastric motility and emptying [38, 39]. CCK is first synthesized as a 115 –amino acid prepro-CCK, which is then subjected to cleavage by prohormone convertases (PCs) and extensive tissue (cell)-specific post-translational processing, leading to the production of several CCK hormones with various sizes (CCK83, CCK58, CCK39, CCK33, CCK22, and CCK8) [40]. For instance, CCK22 and 33 are particularly expressed in the gut and plasma however CCK8 is predominantly presented in neurons [41, 42]. CCK is expressed in many brain areas such as the hippocampus, amygdala, septum, and the hypothalamus [43], where it is co-localized with several neurotransmitters, including γ-aminobutyric acid (GABA), endocannabinoids, dopamine, and serotonin [44 - 46]. CCK receptors (CCK1 or CCK-A and CCK2 or CCK-B) are also expressed in the brain [47, 48]. Numerous studies showed the satiating effects of CCK in various species, including humans, in whom CCK8 reduces meal size and duration, and this anorexigenic effect seems to be mediated by CCK1R [49, 50]. Rodents lacking CCK1R increase their food intake *via* high expression of hypothalamic NPY [51]. Fan and co-workers showed that CCK suppresses food intake *via* activation of POMC and MC4R neurons [52]. Intraperitoneal injection of CCK inhibits AgRP neurons and reduces food intake [53]. Together these data demonstrate the gut-brain communication in short-term regulation of food intake.

### *5.1.3. Glucagon-like Peptide-1 (GLP-1)*

GLP-1 is secreted by L-cells in the distal small intestine and colon, predominantly in response to carbohydrates and fat. GLP-1 is co-localized with oxyntomodulin and peptide YY (PYY). It is cleaved from proglucagon, which is expressed in the gut, pancreas, and brain. GLP-1 is involved in the ileum brake (also known as the distal ileus feedback mechanism), which controls the rate at which food moves through the gut to ensure optimal digestion and absorption [54]. This mechanism results in inhibition of gastroduodenal motility, relaxation of the proximal stomach, inhibition of gastric acid and pancreatic secretion, decreased movement of the food, and decreased food intake [55 - 57]. Several studies have shown that infusion of physiological dose of GLP-1 inhibits food intake in human and in other species [58 - 60]. The anorexigenic effect of GLP-1 is mediated mainly *via* GLP1R as these effects are abolished in GLP1R-deficient mice and are reversed with selective GLP1R antagonists [61]. GLP1R is expressed in many tissues, including the gut, pancreas, hypothalamus, and vagal-afferent nerves [62], which indicate that GLP-1 induces anorexia *via* both vagal and direct central pathways. The vagal effect was shown to be abolished by vagal transaction or deafferentation[1] [63]. GLP-1 can cross the BBB, and activation of GLP1R in the hypothalamus decreases food intake [64]. GLP-1 administration reduces food intake and modulates the hypothalamic expression of NPY, AgRP, POMC, and CART [65].

### *5.1.4. Oxyntomodulin (OXM)*

OXM, like GLP-1, is a 37 amino-acid proglucagon-derived peptide secreted from distal-intestinal L (oxyntic) cells proportionally to ingested calories [66]. OXM decreases gastric acid and pancreatic exocrine secretion and increases intestinal glucose uptake [67 - 69]. The effects of OXM on gastric emptying differ between humans (inhibition following IV infusion) and rodents (no change after acute administration) [70, 71]. OXM inhibits food intake and stimulates energy expenditure in both humans and rodents [72 - 74]. The anorectic effects of OXM are blocked by co-administration of the GLP1R antagonist, extendin$_{9\text{-}39}$, and are not observed in GLP1R knockout mice, indicating that the effect of OXM is mediated by the GLP1R [75]. OXM has been shown to be a dual agonist for GLP1R and glucagon receptor (GCGR) [76]. It has also been shown that the anorectic effect of OXM is mediated *via* αMSH, but not NPY [77].

### *5.1.5. Pancreatic Polypeptide (PP)*

The 36-amino acid anorexigenic peptide PP is mainly synthesized and released from the endocrine pancreas, and to a lesser extent, from the colon and the rectum [78]. Plasma levels of PP are low during fasting status, and increase

proportionally to caloric intake [79]. Peripheral administration of PP reduces food intake in both humans and rodents [80, 81]. Sainsbury and co-workers showed that the anorectic effects of PP are mediated *via* hypothalamic orexin- and brain-derived neurotropic factor (BNDF)-dependent pathways [82]. PP has a higher affinity for the Y4 receptor, and its anorectic effect is abolished in Y4 receptor knockout rodents, indicating that Y4 receptor is a key mediator for PP [83, 84].

### *5.1.6. Peptide YY (PYY)*

Peptide YY is primarily expressed and synthesized in the endocrine L cells in the lower gastrointestinal tract, and to a lesser extent, in the enteric neurons of the stomach and pancreatic endocrine cells [85, 86]. Following a meal, the main circulation form of PYY is the cleaved $PYY_{3-36}$, which rises within 15 minutes, peaks at 90 minutes, and remains high for up to 6 hours and this elevation is proportional to energy intake [87, 88]. The cleavage of PYY to $PYY_{3-36}$ is controlled by the dipeptidyl peptidase IV (DDP-IV). $PYY_{3-36}$ release was observed when the nutrients reach the distal gut, and it is induced by fat-rich diets [89]. In the fasted status, the original PYY form predominates in circulation.

Peripheral administration of $PYY_{3-36}$ reduces food intake in both humans and rodents [90]. Similarly, $PYY_{3-36}$ inhibits food intake in obese subjects, which indicates that obesity is not associated with $PYY_{3-36}$ resistance [91]. Subsequent studies showed that ARC is an important site for $PYY_{3-36}$ action, as a single peripheral administration of $PYY_{3-36}$ induces c-fos gene expression in the hypothalamic ARC. Furthermore, administration of $PYY_{3-36}$ causes a decrease in the hypothalamic NPY gene expression. Electrophysiological studies showed that $PYY_{3-36}$ acts *via* POMC neurons and the release of α-MSH. The anorectic effects of $PYY_{3-36}$ is absent in Y2 receptor knockout mice, indicating a key role of Y2 receptor in mediating $PYY_{3-36}$ action. Together, these data indicate that circulating $PYY_{3-36}$ gains access to the brain (ARC) [92] where it binds to Y2 receptor and increases the activity of anorexigenic POMC/α-MSH neurons whilst decreasing that of orexigenic NPY neurons.

PYY and $PYY_{3-36}$ have profound effects on gastrointestinal motility and secretion (gastric acid secretion, gastric emptying, cephalic phase of gallbladder concentration, and mouth-to-caecum transit), indicating that PYY control food intake *via* the ileal and colonic brakes and the vagus-brainstem-hypothalamic pathway [93 - 95]. Disruption of this pathway has been shown to abolish the anorectic effect of $PYY_{3-36}$. These effects are mediated *via* Y1 receptors on enterocytes, myenteric and submucosal neurons and endothelial cells, Y2 receptors on myenteric and submucosal neurons, extrinsic primary afferent nerve fibers, and Y4 receptors on enterocytes [96 - 98].

### *5.1.7. Amylin*

Amylin, also known as islet amyloid polypeptide (IAPP), is a 37-amino acid pancreatic peptide that is co-secreted with insulin from pancreatic β cells [99]. Peripheral administration of amylin reduces food intake and meal size [100, 101]. It has been shown that calcitonin, an amylin agonist which irreversibly binds to the amylin receptor, is a potent inhibitor of food intake in humans, primates, and rodents [102]. The anorectic effect of amylin is mediated *via* its calcitonin (CTRs)- and receptor activity-modifying proteins (RAMPs)- receptor localized in the brain [103], where amylin interact with hypothalamic neuropeptides such as NPY [104]. In amylin knockout rodents, the density of hypothalamic AgRP-immunoreactive fibers increases, while the density of α-MSH-immunoreactive fibers decreases, indicating that amylin signals also onto AgRP and POMC neurons to suppress food intake [105]. Additionally, it has been reported that amylin phosphorylates the extracellular signal-regulated kinase (ERK) in the area postrema and synergizes with leptin to phosphorylate the signal transducer and activator of transcription 3 (STAT3) in the ARC and VMN to reduce food intake [106, 107].

Amylin has been shown to inhibit gastric secretion, delay gastric emptying, and control gallbladder contraction, but its anorectic effect seems not to be associated with the vagus nerve [12].

### *5.1.8. Enterostatin*

Enterostatin is a pentapeptide cleaved by trypsin from the precursor protein procolipase, which is secreted from the exocrine pancreas in response to ingested fats to facilitate their digestion. Procolipase is also produced in the gastric mucosa of the gastrointestinal tract and several brain areas, including the hypothalamic ARC [108, 109]. Enterostatin reduces food intake, in particular fat intake when given peripherally or centrally [110, 111]. The mechanisms underlying these anorectic effects are complex but seem to involve the F1-ATPase β subunit as the putative enterostatin receptor [112], with downstream signaling pathways including serotonergic and opioidergic systems [113]. Furthermore, Lin and colleagues [114] have shown that the response to enterostatin is also dependent upon MC4R and might be affected partly at least through the regulation of agouti-related protein (AgRP).

### *5.1.9. Apolipoprotein A-IV (ApoA-IV)*

ApoA-IV is a lipid-binding protein, which is primarily synthesized in the small intestine, packaged into chylomicrons, and secreted into intestinal lymph during fat absorption [115]. ApoA-IV has a myriad spectrum of physiological functions

from lipid metabolism, reverse cholesterol transport, glucose metabolism to food intake regulation [116 - 118]. Lo and colleagues have shown that peripheral administration of apoA-IV reduces food intake [119], and this anorectic effect requires CCK and vagus nerve, because apoA-IV could not cross the BBB [120]. Furthermore, both apoA-IV mRNA and proteins are also detected in the hypothalamus, where the signal for regulating food intake and energy homeostasis are integrated [121]. Although the central effect of apoA-IV seems to be mediated *via* phosphoinositide 3-kinase/ (PI3K)/protein kinase B (PKB) pathways [122], the downstream mediators of the peripheral anorectic effects are not well defined. Yan *et al*. [123] have shown that apoA-IV inhibits AgRP/NPY and activates POMC neurons in the ARC, resulting in food intake suppression.

## 5.2. Long-term Regulation of Food Intake

According to the bodyweight set point theory, it is conceivable that embodied energy balance must be a long-term component that, over time (days or weeks), gradually adjusts food intake to maintain body weight (mainly fat content) within a narrow range. Since the 1950's, many scientists have been interested in explaining how the body can estimate the quantity of triglyceride in the fat tissue and thereby regulate the energy intake and fat depot. As adipocytes are directly innervated, Wertheimer and Shapiro [124] suggested that CNS can directly monitor fat content. In 1953, Kennedy advanced the lipostatic theory for the regulation of energy homeostasis, proposing that the hypothalamus could sense humoral information on adiposity degree rather than monitor absolute food intake in rats [125]. Circulating free fatty acids (FFA) and glycerol, as well as adipocyte hypertrophy, were regarded as the signals that inform the hypothalamus about the body's fat (triglycerides) reserves [126]. In 1969, Hervey proposed that the hypothalamus might monitor fat depot *via* blood steroid levels [127]. Baile and colleagues have suggested, in 1973, that prostaglandin might communicate the fat depot status with the hypothalamus [128] based on their data showing that peripheral or central administration of $PGE1^2$ reduces food intake in rodents. Several circulating factors were subsequently proposed but inconclusively proven until the revolutionary discovery of leptin by Friedman's group in 1994 [129].

### *5.2.1. Leptin*

The Obese (Ob) gene was firstly characterized in 1994 in rodents and humans by Friedman's group at the Rockefeller University [129]. It is localized in mouse chromosome 6 and human chromosome 7q31.3. The product, leptin (from the Greek word "leptos" meaning lean" contains 167 amino acids and it is secreted by white adipose tissue proportionally to fat mass [130] and it is thought to signal longer-term energy status [131]. Leptin was found to be also expressed in other

tissues such as placenta, mammary gland, ovary, skeletal muscle, stomach, pituitary gland, and lymphoid tissue (for review see [132]). Peripheral or central administration of leptin has been demonstrated to reduce food intake and body weight [133]. Circulating leptin enters the brain with tanycytes mediation [134] and exerts its anorectic effects through binding to specific leptin receptors located throughout the CNS [135]. Leptin interacts with a complex neural circuit to control appetite and feed intake, activating feeding-related anorexigenic neurons that synthesize POMC and CART, and inhibiting orexigenic neurons that produce NPY and AgRP [136 - 139]. Other hypothalamic mediators of leptin's anorectic action have been found, including melanocortin system. Indeed, leptin receptors are expressed on most POMC neurons in the ARC, and the satiety effect of exogenously administered leptin was reversed by MCR antagonist (SHU9119) pretreatment [140]. Furthermore leptin administration alters the firing rate of ARC POMC neurons in an *ex vivo* electrophysiological slices [141]. ICV administration of leptin inhibits the fasting-induced increase in preproorexin mRNA levels in the rodent hypothalamus [142]. There is considerable evidence indicating that the effect of leptin is also mediated *via* CRF [143]. Leptin has also been reported to inhibit food intake in rodents *via* hypothalamic NUCB2/nesfatin-1 [144]. Central co-injection of leptin and CCK reduced food intake *via* increased hypothalamic CART and thyrotropin-releasing hormone (TRH) [145], indicating that leptin interacts with central CCK and TRH to regulate food intake. Liao and co-workers have shown that leptin targets hypothalamic brain-derived neurotrophic factor (BNDF) to regulate food intake [146].

Overall the ability of leptin to regulate feeding behavior depends on several neuronal populations, neuro-circuits, and neurotransmitters. For instance, leptin receptor is expressed in the median preoptic area (MPO), and its pharmacogenetic activation induces a robust suppression of food intake in rodents [147]. Similarly, administration of leptin in the ventral tegmental dopamine neurons (VTA), where leptin receptor is expressed, inhibits food intake [148]. In the Edinger-Westphal (EW) nucleus, leptin modulates the activity of urocortin 1 neurons and regulates food intake [149]. Scott *et al*. [150] showed that leptin regulates food intake *via* hindbrain GLP-1 neurons. Hayes *et al*. [151] have demonstrated that leptin signaling in the caudal nucleus tractus solitaries and area postrema is required for the regulation of food intake and energy homeostasis. Recent studies suggest that leptin signaling in non-neuronal cells, such as astrocytes, regulates food intake [152]. As summarized in Fig. (**5.1**), leptin interacts with various neurotransmitters in various hypothalamic nuclei (ARC, LHA, MPO, VTA, NTS, *etc*.) to regulate food intake.

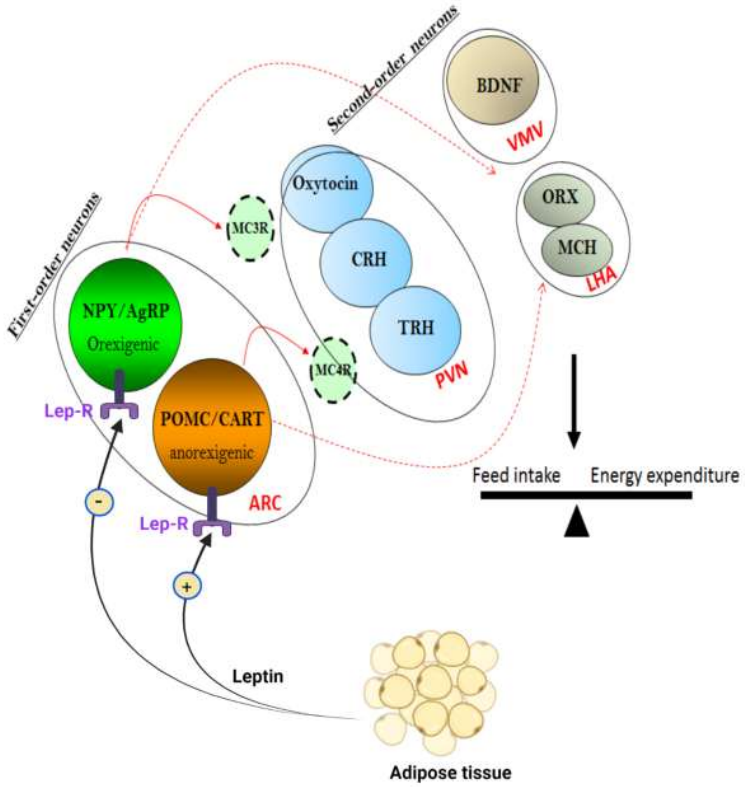


**Fig. (5.1). Leptin and its neuroendocrine circuit in the regulation of energy intake.** Leptin is secreted from adipose tissue, crosses the BBB, and interacts with many important feeding-related hypothalamic neuropeptides and hypothalamic nuclei involved in the control of appetite, including the ARC, VMH, LH, and DMH. AgRP, agouti-related peptide; ARC, arcuate nucleus; CART, cocaine and amphetamine-regulated transcript; CRH, corticotropin-releasing hormone; Lep-R, leptin receptor; LHA, lateral hypothalamus; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; ORX, orexin; PACAP, pituitary adenylate cyclase-activating peptide; POMC, proopiomelanocortin; PVN, paraventricular hypothalamus; SF-1, steroidogenic factor-1; VMH, ventromedial hypothalamus.

Leptin action is mediated by its membrane leptin receptor (LepR), which is expressed in many tissues. The LepR is a single transmembrane-spanning receptor and a member of the cytokine receptor superfamily that includes the gp130 signal-transducing component of the receptors for interleukin 6 (IL-6), granulocyte colony-stimulating factor (G-CSF), and leukemia inhibitory factor (LIF) [153]. In mammals, six LepR isoforms (lepRa-e) were identified (Fig. **5.2**) [154]. Although they all share a common extracellular ligand-binding domain at the N-terminus, the LepR isoforms differ in their intracellular domain and, therefore in their physiologic roles. The soluble LepR (LepRe) lacks the transmembrane domain, and it is possibly involved in clearing leptin from the circulation [155]. The short isoform LepRa is abundantly expressed in the choroid

plexus, and has been hypothesized to be implicated in leptin transport into the CNS through the BBB [156]. The longest isoform LepRb is the only receptor capable of full signal transduction and consequently is essential for leptin action [157].

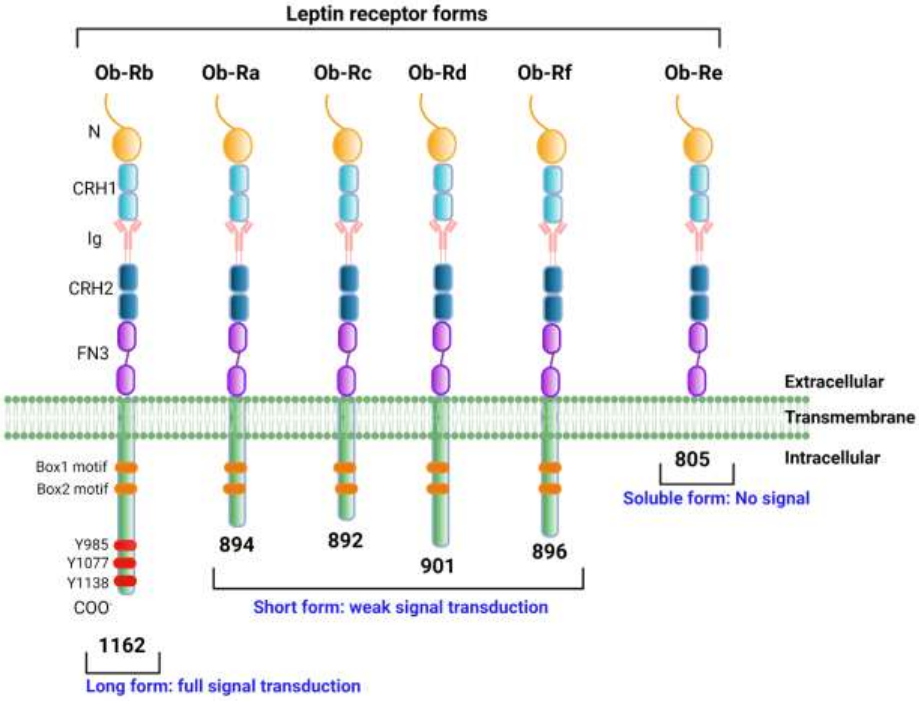


**Fig. (5.2). Schematic representation of leptin receptor forms.** Six spiced isoforms of leptin receptor (Ob-Ra to Ob-Re) have been identified. All the isoforms share identical extracellular ligand-binding domains, but they differ in the intracellular domain length, with the exception of the Ob-Re, which does not have an intracellular domain. The long-form Ob-Rb is the only isoform that contains three tyrosine conserved regions (Y985, Y1077, and Y1138), enabling the leptin-induced activation of the JAK-STAT pathway. Box1 and Box2 are involved in JAK association and activation. CRH1/2, cytokine receptor homology; FN, fibronectin type III domain.

### *5.2.2. Insulin*

The best-known action of insulin is to increase glucose uptake in most peripheral tissues and consequently lower the level of blood glucose. The view that the brain is insensitive to insulin was scattered by the finding that insulin crosses the BBB, enters the brain, reacts with its related receptors on neurons, and triggers various physiological effects. Numerous studies have shown that ICV administration of insulin reduces food intake [158 - 160]. Inhibition of insulin signaling in the brain has an orexigenic effect, which results in increased body weight gain [161, 162]. Mechanistically, the anorexigenic effects of insulin in the CNS are mediated *via* hypothalamic neuropeptides: decrease of orexigenic NPY and increase of

anorexigenic CRH and αMSH [163 - 165]. Because insulin is secreted acutely in response to an increase in blood glucose and because circulating insulin levels are also directly correlated with body fat depots, insulin provides both short-term and long-term homeostatic signals [166 - 168].

To initiate signaling in the CNS, insulin has to reach its receptor. In humans, the insulin receptor (IR) has two subunits, alpha and beta. Two IR (long and short) isoforms are generated by alternative splicing in a tissue-specific manner [169]. The long isoform IR-B is the most prominent isoform in classical insulin-sensitive tissues, including skeletal muscle, liver, and adipose tissue. However, the short isoform IR-A is mainly expressed in the brain [169]. Studies on the presence of IRs in the CNS began in the early 1970s [170]. *In situ* hybridization showed that IR mRNA was the most abundant in the granule cell layers of the olfactory bulb, cerebellum, dentate gyrus, in the pyramidal cell body layers of the piriform cortex, hippocampus, in the choroid plexus, and in the arcuate nucleus of the hypothalamus [171].

Although it is clear that peripheral insulin crosses the BBB and regulates feeding behavior [172], the central production of insulin has also been widely studied. *In situ* hybridization showed the presence of insulin mRNA in the periventricular nucleus of the rat hypothalamus [173]. Several other studies using other techniques have also shown the presence of insulin genes in the mammalian brain [174, 175]. Taken together, these studies indicate that insulin has both peripheral and central effects on energy homeostasis and feeding behavior regulation.

### *5.2.3. Adiponectin*

Adiponectin, also named gelatin-binding protein 28, adipocyte complement related protein 30 (Acrp30), adipose most abundant gene transcript 1 (apM1), or adipoQ, is the most abundant adipokine, which structurally belongs to the complement 1q family. Unlike leptin and other adipose tissue-derived hormones, which circulate at picograms or nanograms per milliliter, adiponectin circulates at levels of micrograms per milliliter [176]. In mammalian plasma, adiponectin can be found as low (dimers or trimers), medium (hexamers), or high (dodecamers) molecular weights. Studies related to the effects of adiponectin on energy intake have yielded controversial results. Indeed, peripheral administration of adiponectin has been reported to reduce body weight by enhancing fatty acid oxidation and energy expenditure without apparent effect on feed intake [177, 178]. However, sustained peripheral expression of transgene adiponectin through a viral vector reduces food intake and body weight [179]. Adiponectin exerts its effects by binding to adiponectin receptors AdipoR1 and AdipoR2. AdipoR1 and AdipoR2 are integral membrane proteins; the N terminus is internal, and the C

terminus is external, which is opposite to the topology of all other reported G protein-coupled receptors [180]. AdipoR1 is a receptor for globular adiponectin, whereas AdipoR2 is a receptor for full-length adiponectin, and AdipoRs may form both homo- and heteromultimers. In rodents, AdipoR1 is ubiquitously expressed and is most abundant in skeletal muscle, whereas AdipoR2 is highly expressed in the liver. Both receptors are also located throughout the CNS, notably in regions of the hypothalamus and brainstem, important in controlling autonomic function and feeding behavior [181]. Fluorescent immunofluorescence analyses showed colocalization of AdipoR1 and AdipoR2 with POMC and NPY neurons [182]. Although adiponectin has been reported to not affect the mRNA expression of NPY/AgRP and POMC/CART [183], its effects seemed to be mediated through CRH and TRH [183, 184]. Circulating adiponectin concentrations decrease in obesity and increase after weight loss [185], and have been found to be inversely correlated to triglyceride levels and visceral fat accumulation [186]. Together these studies indicate that adiponectin is a long-term regulator of energy homeostasis.

### *5.2.4. Visfatin*

Visfatin, also known as a nicotinamide phosphoribosyltransferase (NAMPT), is an adipokine highly expressed in visceral adipose tissue [187]. Two isoforms of Nampt are currently characterized: the intracellular form of Nampt (iNampt), which is the rate limiting-enzyme for $NAD^+$ biosynthesis in mammalian cells, and the extracellular form of Nampt (eNampt), which is considered as a multifunctional cytokine-like molecule that is synthesized and released by adipocytes and other cells exposed to pro-inflammatory stimuli [188]. To date, no specific receptor has been identified for visfatin/eNampt, while some of its actions have been attributed to its intrinsic Nampt enzymatic activity [189]. Recently, Tu *et al*. have shown that visfatin reduces food intake and body weight through activating POMC neurons and the hypothalamic microglia [190].

### *5.2.5. Downstream Signaling Pathways and Feeding*

As stated above, the regulation of food intake and energy balance requires a complex system to homeostatically maintain the body weight set point. The abovementioned adipokines and hormones act through various downstream signaling cascades (Fig. **5.3**). The most known and common intracellular signaling pathway is the Janus Kinase (JAK)-signal transducers and activators of transcription (STAT). In mammals, seven different STAT genes have been identified, STAT1–4, 5A, 5B and 6. Once phosphorylated, STAT molecules form dimers and translocate to the nucleus, where they modify the transcription of target genes. The contribution of STAT in regulating food intake was

demonstrated in a number of transgenic animal models. For instance, mice with a specific deletion of STAT3 from the CNS are obese and hyperphagic [191], demonstrating the necessity of neuronal STAT3 signaling in maintaining normal energy homeostasis. Bates and colleagues developed a transgenic mouse (s/s mouse) in which the phosphorylation site Y1138 that is required for leptin-STAT3 signaling was deleted and showed that the s/s mouse was hyperphagic and obese [192]. In addition, mice with a deletion of STAT3 from either NPY/AgRP or POMC neurons are slightly hyperphagic and mildly obese [193, 194]. Together these studies provided evidence of the critical role of STAT3 in the regulation of food intake, particularly in the role of leptin, which has been confirmed by Buettner *et al*. [195]. STAT3 is also activated by various hormones and cytokines, including insulin [196], adiponectin [197], visfatin [198], tumor necrosis factor-alpha (TNFα) [199], and ciliary neurotrophic factor (CNTF) [200], all of which affects feeding behavior. The activation by numerous cytokines suggests that cytokines may be involved in the suppression of food intake during an immune response [201]. The transcriptional targets involved in food intake regulation by STAT3 include the suppressor of cytokine signaling 3 (SOCS3), POMC and TRH [202 - 204].

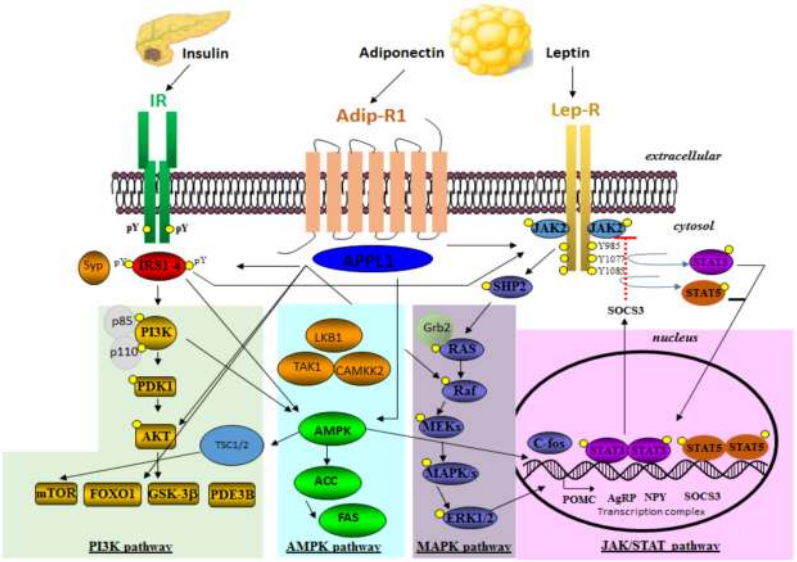


**Fig. (5.3). Cross-talk between leptin, adiponectin, insulin and their downstream signaling pathways in the regulation of food intake**. The scheme summarizes the main intracellular pathways activated by peripheral adipokines. Leptin binds to its receptor Lep-R, which dimerizes and through the activation of JAK2, phosphorylates STAT3 and STAT5 that translocate to the nucleus and modulate transcription of several hypothalamic neuropeptide genes, including NPY, SOCS3 and POMC and regulate food intake. Leptin also acts through components of the insulin signaling cascade since JAK2 phosphorylates IRS proteins and activates PI3K and MAPK signaling pathways. MAPK pathway is also activated by leptin *via*.

Transgenic mice with a conditional deletion of STAT5A and STAT5B in the CNS are hyperphagic and develop severe obesity, indicating that central STAT5 signaling play a crucial role in the regulation of energy homeostasis [205].

Mitogen-activated protein kinase (MAPK) is another signaling pathway downstream of leptin, insulin, and several other adipokines. For instance, extracellular signal-regulated kinase (ERK), a member of the MAPK family, has been shown to be activated by leptin in a receptor-mediated manner involving JAK2 [206]. The activation of ERK1/2 by leptin was restricted to the hypothalamic ARC, and Pharmacological blockade of hypothalamic ERK1/2 reverses the anorectic and weight-reducing effects of leptin [206]. Similarly, PI3K signaling pathway in the brain has been shown to play a pivotal role in the regulation of food intake. Indeed, pretreatment with pharmacological inhibitors of PI3K (wortmannin or LY294002) was effective in blunting the anorectic effect of central injection of leptin [207].

Lep-R-mediated recruitment of SHP2. Insulin binds to its receptor and activates an intrinsic tyrosine kinase, leading to phosphorylation of the intracellular domain of the insulin receptor. IRS proteins bind to the phosphorylated residues on the IR, become activated by tyrosine phosphorylation, and, in turn, initiate downstream cascades such as the activation of the Ras-Raf-MAPK cascade or activation of PI3K pathway including PDK1, GSK3β, and PKB/Akt. SHP2. Adiponectin binds to the extracellular C-terminus of Adip-R1 and recruits APPL1 to the intracellular N-terminus of Adip-R1 and activates leptin/insulin signaling pathways. It is also possible that activation of the AMPK pathway by adiponectin lead to the activation of TSC1/2 signaling that reduce mTOR/S6K-mdiated serine phosphorylation of IRS proteins. This results in the enhancement of IRS tyrosine phosphorylation and insulin signaling. Adip-R, adiponectin receptor; AgRP, agouti-related peptide; Akt, protein kinase B; CAMKK2, $Ca(2^{+})$/calmodulin-dependent protein kinase kinases 2; ERK1/2, extracellular signal-regulated kinase 1/2; FOXO, forkhead transcription factor O; GSK3, glycogen synthase kinase 3; IR, insulin receptor; JAK, janus kinase; Lep-R, leptin receptor; LKB, serine/threonine kinase 11, MAPK, mitogen activated protein kinase; MEK, MAPK kinase; mTOR, mammalian target of rapamycin; PDE3B, phosphodiesterase 3B; PDK, protein-dependent kinase; PI3K, phosphatidylinositol 3 kinase; Ras, Ras small GTPase, SHP2, SH2-domai--containing cytoplasmic tyrosine phosphatase; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TAK, TGFβ activated kinase; TSC, tuberous sclerosis

### 5.3. Hedonic (Non Homeostatic) Regulation of Food Intake

Although the term addiction is applied only to drugs of abuse such as alcohol, cannabis, nicotine, and cocaine, the concept of food addiction has received considerable attention in recent years. One of the definitions of food addiction is "food craving and loss of control over food intake" [208]. Increasing evidence indicates that both drugs of abuse and high palatable foods share common pathways in the limbic system [209]. Increased dopaminergic transmission is one of the key pathways, which occurs *via* direct action on dopaminergic neurons or indirectly through inhibition of GABAergic neurons in the ventral tegmental area (VTA) [210]. Ingestion of sugars and palatable foods is known to induce striatal dopamine release [211], and the dopaminergic system is dysregulated in obese humans and animal models of obesity [212]. Recently, orexin has also been shown to play a key role in mediating drug-induced activation of VTA dopamine neurons and in reward-seeking [213, 214]. Barrot *et al*. [215] showed that decreased activity of VTA cyclic AMP response element-binding protein (CREB) induces the preference for both natural rewards (sucrose) and a drug of abuse (morphine). Similar to exposure of drug of abuse, mice fed a high-fat diet for 4 weeks exhibited a higher expression of delta Fos B [216], which indicate that this gene play a key role in addiction.

## CONCLUSION

Several peripheral peptides controlling food intake have been discovered. These peptides interact in the most complex way with the hypothalamic neuro-circuits. Overall, day-to-day food intake involves the coordination of both homeostatic and hedonic feedback. Although substantial progress has been made, further studies are needed to identify key molecular signatures and effective strategies to prevent/treat metabolic disorders.

## NOTES

[1] Deafferentation: The interruption or destruction of the afferent connections of nerve cells

[2] PGE1: Prostaglandin E1

## REFERENCES

[1] Jéquier E, Tappy L. Regulation of body weight in humans. Physiol Rev 1999; 79(2): 451-80. [http://dx.doi.org/10.1152/physrev.1999.79.2.451] [PMID: 10221987]

[2] Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation 2012; 126(1): 126-32. [http://dx.doi.org/10.1161/CIRCULATIONAHA.111.087213] [PMID: 22753534]

[3] Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. Am J Clin Nutr 2012; 95(4): 989-94.
[http://dx.doi.org/10.3945/ajcn.112.036350] [PMID: 22434603]

[4] Hall KD. Predicting metabolic adaptation, body weight change, and energy intake in humans. Am J Physiol Endocrinol Metab 2010; 298(3): E449-66.
[http://dx.doi.org/10.1152/ajpendo.00559.2009] [PMID: 19934407]

[5] Hall KD, Sacks G, Chandramohan D, *et al.* Quantification of the effect of energy imbalance on bodyweight. Lancet 2011; 378(9793): 826-37.
[http://dx.doi.org/10.1016/S0140-6736(11)60812-X] [PMID: 21872751]

[6] Moran TH, Ladenheim EE. Physiologic and Neural Controls of Eating. Gastroenterol Clin North Am 2016; 45(4): 581-99.
[http://dx.doi.org/10.1016/j.gtc.2016.07.009] [PMID: 27837774]

[7] Brunetti L, Di Nisio C, Orlando G, Ferrante C, Vacca M. The regulation of feeding: a cross talk between peripheral and central signalling. Int J Immunopathol Pharmacol 2005; 18(2): 201-12.
[http://dx.doi.org/10.1177/039463200501800203] [PMID: 15888244]

[8] Lenard NR, Berthoud HR. Central and peripheral regulation of food intake and physical activity: pathways and genes. Obesity (Silver Spring) 2008; 16(S3) (Suppl. 3): S11-22.
[http://dx.doi.org/10.1038/oby.2008.511] [PMID: 19190620]

[9] Zhang Y, Hoon MA, Chandrashekar J, *et al.* Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 2003; 112(3): 293-301.
[http://dx.doi.org/10.1016/S0092-8674(03)00071-0] [PMID: 12581520]

[10] Laugerette F, Passilly-Degrace P, Patris B, *et al.* CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. J Clin Invest 2005; 115(11): 3177-84.
[http://dx.doi.org/10.1172/JCI25299] [PMID: 16276419]

[11] Fox EA. A genetic approach for investigating vagal sensory roles in regulation of gastrointestinal function and food intake. Auton Neurosci 2006; 126-127: 9-29.
[http://dx.doi.org/10.1016/j.autneu.2006.03.005] [PMID: 16677865]

[12] Cummings DE, Overduin J. Gastrointestinal regulation of food intake. J Clin Invest 2007; 117(1): 13-23.
[http://dx.doi.org/10.1172/JCI30227] [PMID: 17200702]

[13] Woods SC, Lutz TA, Geary N, Langhans W. Pancreatic signals controlling food intake; insulin, glucagon and amylin. Philos Trans R Soc Lond B Biol Sci 2006; 361(1471): 1219-35.
[http://dx.doi.org/10.1098/rstb.2006.1858] [PMID: 16815800]

[14] Novin D, Robinson K, Culbreth LA, Tordoff MG. Is there a role for the liver in the control of food intake? Am J Clin Nutr 1985; 42(5) (Suppl.): 1050-62.
[http://dx.doi.org/10.1093/ajcn/42.5.1050] [PMID: 4061358]

[15] Grannell A, De Vito G, Murphy JC, le Roux CW. The influence of skeletal muscle on appetite regulation. Expert Rev Endocrinol Metab 2019; 14(4): 267-82.
[http://dx.doi.org/10.1080/17446651.2019.1618185] [PMID: 31106601]

[16] Henry BA, Clarke IJ. Adipose tissue hormones and the regulation of food intake. J Neuroendocrinol 2008; 20(6): 842-9.
[http://dx.doi.org/10.1111/j.1365-2826.2008.1730.x] [PMID: 18601708]

[17] Trayhurn P, Bing C. Appetite and energy balance signals from adipocytes. Philos Trans R Soc Lond B Biol Sci 2006; 361(1471): 1237-49.
[http://dx.doi.org/10.1098/rstb.2006.1859] [PMID: 16815801]

[18] Havel PJ. Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. Exp Biol Med (Maywood) 2001; 226(11): 963-77.

[http://dx.doi.org/10.1177/153537020122601102] [PMID: 11743131]

[19] Van Itallie TB, Smith NS, Quartermain DP. Short-term and long-term components in the regulation of food intake: evidence for a modulatory role of carbohydrate status. Am J Clin Nutr 1977; 30(5): 742-57.
[http://dx.doi.org/10.1093/ajcn/30.5.742] [PMID: 855815]

[20] Moran TH. Gut peptides in the control of food intake. Int J Obes 2009; 33(S1) (Suppl. 1): S7-S10.
[http://dx.doi.org/10.1038/ijo.2009.9] [PMID: 19363513]

[21] Camilleri M. Integrated upper gastrointestinal response to food intake. Gastroenterology 2006; 131(2): 640-58.
[http://dx.doi.org/10.1053/j.gastro.2006.03.023] [PMID: 16890616]

[22] Faulconbridge LF, Cummings DE, Kaplan JM, Grill HJ. Hyperphagic effects of brainstem ghrelin administration. Diabetes 2003; 52(9): 2260-5.
[http://dx.doi.org/10.2337/diabetes.52.9.2260] [PMID: 12941764]

[23] Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000; 407(6806): 908-13.
[http://dx.doi.org/10.1038/35038090] [PMID: 11057670]

[24] Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001; 50(8): 1714-9.
[http://dx.doi.org/10.2337/diabetes.50.8.1714] [PMID: 11473029]

[25] Bagnasco M, Kalra PS, Kalra SP. Ghrelin and leptin pulse discharge in fed and fasted rats. Endocrinology 2002; 143(2): 726-9.
[http://dx.doi.org/10.1210/endo.143.2.8743] [PMID: 11796530]

[26] Saito E, Kaiya H, Takagi T, *et al.* Chicken ghrelin and growth hormone-releasing peptide-2 inhibit food intake of neonatal chicks. Eur J Pharmacol 2002; 453(1): 75-9.
[http://dx.doi.org/10.1016/S0014-2999(02)02393-2] [PMID: 12393062]

[27] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormon--releasing acylated peptide from stomach. Nature 1999; 402(6762): 656-60.
[http://dx.doi.org/10.1038/45230] [PMID: 10604470]

[28] Kaiya H, van der Geyten S, Kojima M, *et al.* Chicken ghrelin: purification, cDNA cloning, and biological activity. Endocrinology 2002; 143(9): 3454-63.
[http://dx.doi.org/10.1210/en.2002-220255] [PMID: 12193558]

[29] Gnanapavan S, Kola B, Bustin SA, *et al.* The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002; 87(6): 2988-91.
[http://dx.doi.org/10.1210/jcem.87.6.8739] [PMID: 12050285]

[30] Burdyga G, Varro A, Dimaline R, Thompson DG, Dockray GJ. Ghrelin receptors in rat and human nodose ganglia: putative role in regulating CB-1 and MCH receptor abundance. Am J Physiol Gastrointest Liver Physiol 2006; 290(6): G1289-97.
[http://dx.doi.org/10.1152/ajpgi.00543.2005] [PMID: 16423919]

[31] Willesen MG, Kristensen P, Rømer J. Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. Neuroendocrinology 1999; 70(5): 306-16.
[http://dx.doi.org/10.1159/000054491] [PMID: 10567856]

[32] Lawrence CB, Snape AC, Baudoin FMH, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology 2002; 143(1): 155-62.
[http://dx.doi.org/10.1210/endo.143.1.8561] [PMID: 11751604]

[33] Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 2000; 141(12): 4797-800.
[http://dx.doi.org/10.1210/endo.141.12.7920] [PMID: 11108296]

[34] Chen HY, Trumbauer ME, Chen AS, *et al.* Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 2004; 145(6): 2607-12. [http://dx.doi.org/10.1210/en.2003-1596] [PMID: 14962995]

[35] Seoane LM, López M, Tovar S, Casanueva FF, Señarís R, Diéguez C. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. Endocrinology 2003; 144(2): 544-51. [http://dx.doi.org/10.1210/en.2002-220795] [PMID: 12538615]

[36] Shintani M, Ogawa Y, Ebihara K, *et al.* Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 2001; 50(2): 227-32. [http://dx.doi.org/10.2337/diabetes.50.2.227] [PMID: 11272130]

[37] Wren AM, Small CJ, Fribbens CV, *et al.* The hypothalamic mechanisms of the hypophysiotropic action of ghrelin. Neuroendocrinology 2002; 76(5): 316-24. [http://dx.doi.org/10.1159/000066629] [PMID: 12457042]

[38] Grider JR. Role of cholecystokinin in the regulation of gastrointestinal motility. J Nutr 1994; 124(8) (Suppl. 8): 1334S-9S. [http://dx.doi.org/10.1093/jn/124.suppl_8.1334S] [PMID: 8064380]

[39] Burckhardt B, Delco F, Ensinck JW, *et al.* Cholecystokinin is a physiological regulator of gastric acid secretion in man. Eur J Clin Invest 1994; 24(6): 370-6. [http://dx.doi.org/10.1111/j.1365-2362.1994.tb02178.x] [PMID: 7957488]

[40] Cain BM, Connolly K, Blum A, Vishnuvardham D, Marchand JE, Beinfeld MC. Distribution and colocalization of cholecystokinin with the prohormone convertase enzymes PC1, PC2, and PC5 in rat brain. J Comp Neurol 2003; 467(3): 307-25. [http://dx.doi.org/10.1002/cne.10924] [PMID: 14608596]

[41] Dockray GJ. Cholecystokinins in rat cerebral cortex: Identification, purification and characterization by immunochemical methods. Brain Res 1980; 188(1): 155-65. [http://dx.doi.org/10.1016/0006-8993(80)90564-8] [PMID: 7370750]

[42] Vanderhaeghen JJ, Signeau JC, Gepts W. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 1975; 257(5527): 604-5. [http://dx.doi.org/10.1038/257604a0] [PMID: 1165787]

[43] Beinfeld MC, Meyer DK, Eskay RL, Jensen RT, Brownstein MJ. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. Brain Res 1981; 212(1): 51-7. [http://dx.doi.org/10.1016/0006-8993(81)90031-7] [PMID: 7225864]

[44] Hökfelt T, Rehfeld JF, Skirboll L, Ivemark B, Goldstein M, Markey K. Evidence for coexistence of dopamine and CCK in meso-limbic neurones. Nature 1980; 285(5765): 476-8. [http://dx.doi.org/10.1038/285476a0] [PMID: 6105617]

[45] Somogyi P, Hodgson AJ, Smith AD, Nunzi MG, Gorio A, Wu JY. Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin- immunoreactive material. J Neurosci 1984; 4(10): 2590-603. [http://dx.doi.org/10.1523/JNEUROSCI.04-10-02590.1984] [PMID: 6149275]

[46] Kosaka T, Kosaka K, Tateishi K, *et al.* GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. J Comp Neurol 1985; 239(4): 420-30. [http://dx.doi.org/10.1002/cne.902390408] [PMID: 2413092]

[47] Innis RB, Snyder SH. Distinct cholecystokinin receptors in brain and pancreas. Proc Natl Acad Sci USA 1980; 77(11): 6917-21. [http://dx.doi.org/10.1073/pnas.77.11.6917] [PMID: 6256771]

[48] Honda T, Wada E, Battey JF, Wank SA. Differential Gene Expression of CCKA and CCKB Receptors

in the Rat Brain. Mol Cell Neurosci 1993; 4(2): 143-54.
[http://dx.doi.org/10.1006/mcne.1993.1018] [PMID: 19912917]

[49] Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr 1981; 34(2): 154-60.
[http://dx.doi.org/10.1093/ajcn/34.2.154] [PMID: 6259918]

[50] Kopin AS, Mathes WF, McBride EW, *et al.* The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. J Clin Invest 1999; 103(3): 383-91.
[http://dx.doi.org/10.1172/JCI4901] [PMID: 9927499]

[51] Moran TH, Bi S. Hyperphagia and obesity in OLETF rats lacking CCK-1 receptors. Philos Trans R Soc Lond B Biol Sci 2006; 361(1471): 1211-8.
[http://dx.doi.org/10.1098/rstb.2006.1857] [PMID: 16815799]

[52] Fan W, Ellacott KLJ, Halatchev IG, Takahashi K, Yu P, Cone RD. Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. Nat Neurosci 2004; 7(4): 335-6.
[http://dx.doi.org/10.1038/nn1214] [PMID: 15034587]

[53] Beutler LR, Chen Y, Ahn JS, Lin YC, Essner RA, Knight ZA. Dynamics of Gut-Brain Communication Underlying Hunger. Neuron 2017; 96(2): 461-475.e5.
[http://dx.doi.org/10.1016/j.neuron.2017.09.043] [PMID: 29024666]

[54] van Avesaat M, Troost FJ, Ripken D, Hendriks HF, Masclee A A M. Ileal brake activation: macronutrient-specific effects on eating behavior? Int J Obes 2015; 39(2): 235-43.
[http://dx.doi.org/10.1038/ijo.2014.112] [PMID: 24957485]

[55] Maljaars PWJ, Peters HPF, Mela DJ, Masclee AAM. Ileal brake: A sensible food target for appetite control. A review. Physiol Behav 2008; 95(3): 271-81.
[http://dx.doi.org/10.1016/j.physbeh.2008.07.018] [PMID: 18692080]

[56] Schirra J, Nicolaus M, Roggel R, *et al.* Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut 2006; 55(2): 243-51.
[http://dx.doi.org/10.1136/gut.2004.059741] [PMID: 15985560]

[57] Schjoldager BT, Mortensen PE, Christiansen J, Orskov C, Holst JJ. GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. Dig Dis Sci 1989; 34(5): 703-8.
[http://dx.doi.org/10.1007/BF01540341] [PMID: 2714145]

[58] Verdich C, Flint A, Gutzwiller JP, *et al.* A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. J Clin Endocrinol Metab 2001; 86(9): 4382-9.
[PMID: 11549680]

[59] Turton MD, O'Shea D, Gunn I, *et al.* A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 1996; 379(6560): 69-72.
[http://dx.doi.org/10.1038/379069a0] [PMID: 8538742]

[60] Donahey JCK, van Dijk G, Woods SC, Seeley RJ. Intraventricular GLP-1 reduces short- but not long-term food intake or body weight in lean and obese rats. Brain Res 1998; 779(1-2): 75-83.
[http://dx.doi.org/10.1016/S0006-8993(97)01057-3] [PMID: 9473596]

[61] Baggio LL, Huang Q, Brown TJ, Drucker DJ. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. Gastroenterology 2004; 127(2): 546-58.
[http://dx.doi.org/10.1053/j.gastro.2004.04.063] [PMID: 15300587]

[62] Drucker DJ. The biology of incretin hormones. Cell Metab 2006; 3(3): 153-65.
[http://dx.doi.org/10.1016/j.cmet.2006.01.004] [PMID: 16517403]

[63] Abbott CR, Monteiro M, Small CJ, *et al.* The inhibitory effects of peripheral administration of peptide YY3–36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal–brainstem–hypothalamic pathway. Brain Res 2005; 1044(1): 127-31.

[http://dx.doi.org/10.1016/j.brainres.2005.03.011] [PMID: 15862798]

[64] Kinzig KP, D'Alessio DA, Seeley RJ. The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. J Neurosci 2002; 22(23): 10470-6. [http://dx.doi.org/10.1523/JNEUROSCI.22-23-10470.2002] [PMID: 12451146]

[65] Seo S, Ju S, Chung H, Lee D, Park S. Acute effects of glucagon-like peptide-1 on hypothalamic neuropeptide and AMP activated kinase expression in fasted rats. Endocr J 2008; 55(5): 867-74. [http://dx.doi.org/10.1507/endocrj.K08E-091] [PMID: 18506089]

[66] Le Quellec A, Kervran A, Blache P, Ciurana AJ, Bataille D. Oxyntomodulin-like immunoreactivity: diurnal profile of a new potential enterogastrone. J Clin Endocrinol Metab 1992; 74(6): 1405-9. [PMID: 1592887]

[67] Biedzinski TM, Bataille D, Devaux MA, Sarles H. The effect of oxyntomodulin (Glucagon-37) and glucagon on exocrine pancreatic secretion in the conscious rat. Peptides 1987; 8(6): 967-72. [http://dx.doi.org/10.1016/0196-9781(87)90122-7] [PMID: 3441447]

[68] Anini Y, Jarrousse C, Chariot J, *et al.* Oxyntomodulin inhibits pancreatic secretion through the nervous system in rats. Pancreas 2000; 20(4): 348-60. [http://dx.doi.org/10.1097/00006676-200005000-00003] [PMID: 10824688]

[69] Collie NL, Zhu Z, Jordan S, Reeve JR Jr. Oxyntomodulin stimulates intestinal glucose uptake in rats. Gastroenterology 1997; 112(6): 1961-70. [http://dx.doi.org/10.1053/gast.1997.v112.pm9178688] [PMID: 9178688]

[70] Maida A, Lovshin JA, Baggio LL, Drucker DJ. The glucagon-like peptide-1 receptor agonist oxyntomodulin enhances beta-cell function but does not inhibit gastric emptying in mice. Endocrinology 2008; 149(11): 5670-8. [http://dx.doi.org/10.1210/en.2008-0336] [PMID: 18669601]

[71] Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Oxyntomodulin from distal gut. Dig Dis Sci 1989; 34(9): 1411-9. [http://dx.doi.org/10.1007/BF01538078] [PMID: 2670487]

[72] Cohen MA, Ellis SM, Le Roux CW, *et al.* Oxyntomodulin suppresses appetite and reduces food intake in humans. J Clin Endocrinol Metab 2003; 88(10): 4696-701. [http://dx.doi.org/10.1210/jc.2003-030421] [PMID: 14557443]

[73] Wynne K, Park AJ, Small CJ, *et al.* Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. Diabetes 2005; 54(8): 2390-5. [http://dx.doi.org/10.2337/diabetes.54.8.2390] [PMID: 16046306]

[74] Dakin CL, Gunn I, Small CJ, *et al.* Oxyntomodulin inhibits food intake in the rat. Endocrinology 2001; 142(10): 4244-50. [http://dx.doi.org/10.1210/endo.142.10.8430] [PMID: 11564680]

[75] Wynne K, Field BC, Bloom SR. The mechanism of action for oxyntomodulin in the regulation of obesity. Curr Opin Investig Drugs 2010; 11(10): 1151-7. [PMID: 20872318]

[76] Gros L, Thorens B, Bataille D, Kervran A. Glucagon-like peptide-1-(7-36) amide, oxyntomodulin, and glucagon interact with a common receptor in a somatostatin-secreting cell line. Endocrinology 1993; 133(2): 631-8. [http://dx.doi.org/10.1210/endo.133.2.8102095] [PMID: 8102095]

[77] Dakin CL, Small CJ, Batterham RL, *et al.* Peripheral oxyntomodulin reduces food intake and body weight gain in rats. Endocrinology 2004; 145(6): 2687-95. [http://dx.doi.org/10.1210/en.2003-1338] [PMID: 15001546]

[78] Adrian TE, Bloom SR, Bryant MG, Polak JM, Heitz PH, Barnes AJ. Distribution and release of human pancreatic polypeptide. Gut 1976; 17(12): 940-4. [http://dx.doi.org/10.1136/gut.17.12.940] [PMID: 828120]

[79] Track NS, McLeod RS, Mee AV. Human pancreatic polypeptide: studies of fasting and postprandial plasma concentrations. Can J Physiol Pharmacol 1980; 58(12): 1484-9.
[http://dx.doi.org/10.1139/y80-223] [PMID: 7237243]

[80] Batterham RL, Le Roux CW, Cohen MA, *et al.* Pancreatic polypeptide reduces appetite and food intake in humans. J Clin Endocrinol Metab 2003; 88(8): 3989-92.
[http://dx.doi.org/10.1210/jc.2003-030630] [PMID: 12915697]

[81] Malaisse-Lagae F, Carpentier JL, Patel YC, Malaisse WJ, Orci L. Pancreatic polypeptide: A possible role in the regulation of food intake in the mouse. Hypothesis. Experientia 1977; 33(7): 915-7.
[http://dx.doi.org/10.1007/BF01951279] [PMID: 891771]

[82] Sainsbury A, Shi YC, Zhang L, *et al.* Y4 receptors and pancreatic polypeptide regulate food intake *via* hypothalamic orexin and brain-derived neurotropic factor dependent pathways. Neuropeptides 2010; 44(3): 261-8.
[http://dx.doi.org/10.1016/j.npep.2010.01.001] [PMID: 20116098]

[83] Blomqvist AG, Herzog H. Y-receptor subtypes—how many more? Trends Neurosci 1997; 20(7): 294-8.
[http://dx.doi.org/10.1016/S0166-2236(96)01057-0] [PMID: 9223221]

[84] Balasubramaniam A, Mullins DE, Lin S, *et al.* Neuropeptide Y (NPY) Y4 receptor selective agonists based on NPY(32-36): development of an anorectic Y4 receptor selective agonist with picomolar affinity. J Med Chem 2006; 49(8): 2661-5.
[http://dx.doi.org/10.1021/jm050907d] [PMID: 16610810]

[85] Ekblad E, Sundler F. Distribution of pancreatic polypeptide and peptide YY. Peptides 2002; 23(2): 251-61.
[http://dx.doi.org/10.1016/S0196-9781(01)00601-5] [PMID: 11825640]

[86] Cox HM, Peptide YY. Peptide YY: A neuroendocrine neighbor of note. Peptides 2007; 28(2): 345-51.
[http://dx.doi.org/10.1016/j.peptides.2006.07.023] [PMID: 17194503]

[87] McGowan BMC, Bloom SR. Peptide YY and appetite control. Curr Opin Pharmacol 2004; 4(6): 583-8.
[http://dx.doi.org/10.1016/j.coph.2004.06.007] [PMID: 15525548]

[88] Adrian TE, Ferri G-L, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology 1985; 89(5): 1070-7.
[http://dx.doi.org/10.1016/0016-5085(85)90211-2] [PMID: 3840109]

[89] Essah PA, Levy JR, Sistrun SN, Kelly SM, Nestler JE. Effect of macronutrient composition on postprandial peptide YY levels. J Clin Endocrinol Metab 2007; 92(10): 4052-5.
[http://dx.doi.org/10.1210/jc.2006-2273] [PMID: 17726080]

[90] Batterham RL, Cowley MA, Small CJ, *et al.* Gut hormone PYY3-36 physiologically inhibits food intake. Nature 2002; 418(6898): 650-4.
[http://dx.doi.org/10.1038/nature00887] [PMID: 12167864]

[91] Batterham RL, Cohen MA, Ellis SM, *et al.* Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med 2003; 349(10): 941-8.
[http://dx.doi.org/10.1056/NEJMoa030204] [PMID: 12954742]

[92] Nonaka N, Shioda S, Niehoff ML, Banks WA. Characterization of blood-brain barrier permeability to PYY3-36 in the mouse. J Pharmacol Exp Ther 2003; 306(3): 948-53.
[http://dx.doi.org/10.1124/jpet.103.051821] [PMID: 12750431]

[93] Pironi L, Stanghellini V, Miglioli M, *et al.* Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. Gastroenterology 1993; 105(3): 733-9.
[http://dx.doi.org/10.1016/0016-5085(93)90890-O] [PMID: 8359644]

[94] Lin HC, Zhao XT, Wang L, Wong H. Fat-induced ileal brake in the dog depends on peptide YY.

Gastroenterology 1996; 110(5): 1491-5.
[http://dx.doi.org/10.1053/gast.1996.v110.pm8613054] [PMID: 8613054]

[95] Nightingale JM, Kamm MA, van der Sijp JR, Ghatei MA, Bloom SR, Lennard-Jones JE. Gastrointestinal hormones in short bowel syndrome. Peptide YY may be the 'colonic brake' to gastric emptying. Gut 1996; 39(2): 267-72.
[http://dx.doi.org/10.1136/gut.39.2.267] [PMID: 8977342]

[96] Field BCT, Chaudhri OB, Bloom SR. Bowels control brain: gut hormones and obesity. Nat Rev Endocrinol 2010; 6(8): 444-53.
[http://dx.doi.org/10.1038/nrendo.2010.93] [PMID: 20585346]

[97] Tough IR, Forbes S, Tolhurst R, *et al.* Endogenous peptide YY and neuropeptide Y inhibit colonic ion transport, contractility and transit differentially via $Y_1$ and $Y_2$ receptors. Br J Pharmacol 2011; 164(2b): 471-84.
[http://dx.doi.org/10.1111/j.1476-5381.2011.01401.x] [PMID: 21457230]

[98] Cox HM, Tough IR, Neuropeptide Y. Neuropeptide Y, $Y_1$, $Y_2$ and $Y_4$ receptors mediate Y agonist responses in isolated human colon mucosa. Br J Pharmacol 2002; 135(6): 1505-12.
[http://dx.doi.org/10.1038/sj.bjp.0704604] [PMID: 11906964]

[99] Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB. Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci USA 1987; 84(23): 8628-32.
[http://dx.doi.org/10.1073/pnas.84.23.8628] [PMID: 3317417]

[100] Lutz TA. Amylinergic control of food intake. Physiol Behav 2006; 89(4): 465-71.
[http://dx.doi.org/10.1016/j.physbeh.2006.04.001] [PMID: 16697020]

[101] Morley JE, Flood JF, Horowitz M, Morley PM, Walter MJ. Modulation of food intake by peripherally administered amylin. Am J Physiol 1994; 267(1 Pt 2): R178-84.
[PMID: 8048622]

[102] Hilton JM, Dowton M, Houssami S, Sexton PM. Identification of key components in the irreversibility of salmon calcitonin binding to calcitonin receptors. J Endocrinol 2000; 166(1): 213-26.
[http://dx.doi.org/10.1677/joe.0.1660213] [PMID: 10856900]

[103] Beaumont K, Kenney MA, Young AA, Rink TJ. High affinity amylin binding sites in rat brain. Mol Pharmacol 1993; 44(3): 493-7.
[PMID: 8396712]

[104] Morris MJ, Nguyen T. Does neuropeptide Y contribute to the anorectic action of amylin? Peptides 2001; 22(3): 541-6.
[http://dx.doi.org/10.1016/S0196-9781(01)00348-5] [PMID: 11287112]

[105] Lutz TA, Coester B, Whiting L, *et al.* Amylin Selectively Signals Onto POMC Neurons in the Arcuate Nucleus of the Hypothalamus. Diabetes 2018; 67(5): 805-17.
[http://dx.doi.org/10.2337/db17-1347] [PMID: 29467172]

[106] Turek VF, Trevaskis JL, Levin BE, *et al.* Mechanisms of amylin/leptin synergy in rodent models. Endocrinology 2010; 151(1): 143-52.
[http://dx.doi.org/10.1210/en.2009-0546] [PMID: 19875640]

[107] Potes CS, Boyle CN, Wookey PJ, Riediger T, Lutz TA. Involvement of the extracellular signal-regulated kinase 1/2 signaling pathway in amylin's eating inhibitory effect. Am J Physiol Regul Integr Comp Physiol 2012; 302(3): R340-51.
[http://dx.doi.org/10.1152/ajpregu.00380.2011] [PMID: 22129618]

[108] Koizumi M, Nakanishi Y, Sato H, Morinaga Y, Ido T, Kimura S. Uptake across the blood–brain barrier and tissue distribution of enterostatin after peripheral administration in rats. Physiol Behav 2002; 77(1): 5-10.
[http://dx.doi.org/10.1016/S0031-9384(02)00805-3] [PMID: 12213496]

[109] York DA, Lin L, Thomas SR, Braymer HD, Park M. Procolipase gene expression in the rat brain: Source of endogenous enterostatin production in the brain. Brain Res 2006; 1087(1): 52-9.
[http://dx.doi.org/10.1016/j.brainres.2006.03.013] [PMID: 16624258]

[110] Erlanson-Albertsson C, Yorkf D. Enterostatin--a peptide regulating fat intake. Obes Res 1997; 5(4): 360-72.
[http://dx.doi.org/10.1002/j.1550-8528.1997.tb00565.x] [PMID: 9285845]

[111] Erlanson-Albertsson C, Larsson A. The activation peptide of pancreatic procolipase decreases food intake in rats. Regul Pept 1988; 22(4): 325-31.
[http://dx.doi.org/10.1016/0167-0115(88)90109-7] [PMID: 3187077]

[112] Park M, Lin L, Thomas S, *et al.* The F1-ATPase β-subunit is the putative enterostatin receptor. Peptides 2004; 25(12): 2127-33.
[http://dx.doi.org/10.1016/j.peptides.2004.08.022] [PMID: 15572201]

[113] Koizumi M, Kimura S. Enterostatin increases extracellular serotonin and dopamine in the lateral hypothalamic area in rats measured by *in vivo* microdialysis. Neurosci Lett 2002; 320(1-2): 96-8.
[http://dx.doi.org/10.1016/S0304-3940(01)02580-0] [PMID: 11849772]

[114] Lin L, Park M, York DA. Enterostatin inhibition of dietary fat intake is modulated through the melanocortin system. Peptides 2007; 28(3): 643-9.
[http://dx.doi.org/10.1016/j.peptides.2006.10.003] [PMID: 17113194]

[115] Green PHR, Glickman RM, Riley JW, Quinet E. Human Apolipoprotein A-IV. J Clin Invest 1980; 65(4): 911-9.
[http://dx.doi.org/10.1172/JCI109745] [PMID: 6987270]

[116] Goldberg IJ, Scheraldi CA, Yacoub LK, Saxena U, Bisgaier CL. Lipoprotein ApoC-II activation of lipoprotein lipase. Modulation by apolipoprotein A-IV. J Biol Chem 1990; 265(8): 4266-72.
[http://dx.doi.org/10.1016/S0021-9258(19)39557-2] [PMID: 2307668]

[117] Stein O, Stein Y, Lefevre M, Roheim PS. The role of apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and liposomes derived from an ether analog of phosphatidylcholine. Biochim Biophys Acta Lipids Lipid Metab 1986; 878(1): 7-13.
[http://dx.doi.org/10.1016/0005-2760(86)90337-1] [PMID: 3089295]

[118] Wang F, Kohan AB, Kindel TL, *et al.* Apolipoprotein A-IV improves glucose homeostasis by enhancing insulin secretion. Proc Natl Acad Sci USA 2012; 109(24): 9641-6.
[http://dx.doi.org/10.1073/pnas.1201433109] [PMID: 22619326]

[119] Lo CC, Langhans W, Georgievsky M, *et al.* Apolipoprotein AIV requires cholecystokinin and vagal nerves to suppress food intake. Endocrinology 2012; 153(12): 5857-65.
[http://dx.doi.org/10.1210/en.2012-1427] [PMID: 23027805]

[120] Shen L, Pearson KJ, Xiong Y, *et al.* Characterization of apolipoprotein A-IV in brain areas involved in energy homeostasis. Physiol Behav 2008; 95(1-2): 161-7.
[http://dx.doi.org/10.1016/j.physbeh.2008.05.022] [PMID: 18577393]

[121] Liu M, Doi T, Shen L, *et al.* Intestinal satiety protein apolipoprotein AIV is synthesized and regulated in rat hypothalamus. Am J Physiol Regul Integr Comp Physiol 2001; 280(5): R1382-7.
[http://dx.doi.org/10.1152/ajpregu.2001.280.5.R1382] [PMID: 11294757]

[122] Shen L, Lo CC, Woollett LA, Liu M. Apolipoprotein A-IV exerts its anorectic action through a PI3K/Akt signaling pathway in the hypothalamus. Biochem Biophys Res Commun 2017; 494(1-2): 152-7.
[http://dx.doi.org/10.1016/j.bbrc.2017.10.063] [PMID: 29037812]

[123] Yan C, He Y, Xu Y, *et al.* Apolipoprotein A-IV Inhibits AgRP/NPY Neurons and Activates Pro-Opiomelanocortin Neurons in the Arcuate Nucleus. Neuroendocrinology 2016; 103(5): 476-88.
[http://dx.doi.org/10.1159/000439436] [PMID: 26337236]

[124] Wertheimer E, Shapiro B. The physiology of adipose tissue. Physiol Rev 1948; 28(4): 451-64. [http://dx.doi.org/10.1152/physrev.1948.28.4.451] [PMID: 18894958]

[125] Kennedy GC. The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Soc Lond B Biol Sci 1953; 140(901): 578-92. [http://dx.doi.org/10.1098/rspb.1953.0009] [PMID: 13027283]

[126] Bray GA, Campfield LA. Metabolic factors in the control of energy stores. Metabolism 1975; 24(1): 99-117. [http://dx.doi.org/10.1016/0026-0495(75)90011-6] [PMID: 1089189]

[127] Hervey GR. Regulation of energy balance. Nature 1969; 222(5194): 629-31. [http://dx.doi.org/10.1038/222629a0] [PMID: 5768271]

[128] Baile C, Waynesimpson C, Markbean S, McLaughlin C, Jacobs H. Prostaglandins and food intake of rats: A component of energy balance regulation? Physiol Behav 1973; 10(6): 1077-85. [http://dx.doi.org/10.1016/0031-9384(73)90191-1] [PMID: 4197924]

[129] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372(6505): 425-32. [http://dx.doi.org/10.1038/372425a0] [PMID: 7984236]

[130] Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334(5): 292-5. [http://dx.doi.org/10.1056/NEJM199602013340503] [PMID: 8532024]

[131] Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995; 269(5223): 546-9. [http://dx.doi.org/10.1126/science.7624778] [PMID: 7624778]

[132] Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. Int J Obes 2002; 26(11): 1407-33. [http://dx.doi.org/10.1038/sj.ijo.0802142] [PMID: 12439643]

[133] Weigle DS, Bukowski TR, Foster DC, *et al.* Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. J Clin Invest 1995; 96(4): 2065-70. [http://dx.doi.org/10.1172/JCI118254] [PMID: 7560100]

[134] Balland E, Dam J, Langlet F, *et al.* Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. Cell Metab 2014; 19(2): 293-301. [http://dx.doi.org/10.1016/j.cmet.2013.12.015] [PMID: 24506870]

[135] Elmquist JK, Bjørbaek C, Ahima RS, Flier JS, Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. J Comp Neurol 1998; 395(4): 535-47. [http://dx.doi.org/10.1002/(SICI)1096-9861(19980615)395:4<535::AID-CNE9>3.0.CO;2-2] [PMID: 9619505]

[136] Schwartz MW, Baskin DG, Bukowski TR, *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996; 45(4): 531-5. [http://dx.doi.org/10.2337/diab.45.4.531] [PMID: 8603777]

[137] Stephens TW, Basinski M, Bristow PK, *et al.* The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995; 377(6549): 530-2. [http://dx.doi.org/10.1038/377530a0] [PMID: 7566151]

[138] Schwartz MW, Seeley RJ, Woods SC, *et al.* Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 1997; 46(12): 2119-23. [http://dx.doi.org/10.2337/diab.46.12.2119] [PMID: 9392508]

[139] Elmquist JK, Maratos-Flier E, Saper CB, Flier JS. Unraveling the central nervous system pathways underlying responses to leptin. Nat Neurosci 1998; 1(6): 445-50. [http://dx.doi.org/10.1038/2164] [PMID: 10196541]

[140] Seeley RJ, Yagaloff KA, Fisher SL, *et al.* Melanocortin receptors in leptin effects. Nature 1997; 390(6658): 349.
[http://dx.doi.org/10.1038/37016] [PMID: 9389472]

[141] Cowley MA, Smart JL, Rubinstein M, *et al.* Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 2001; 411(6836): 480-4.
[http://dx.doi.org/10.1038/35078085] [PMID: 11373681]

[142] López M, Seoane L, del Carmen García M, *et al.* Leptin regulation of prepro-orexin and orexin receptor mRNA levels in the hypothalamus. Biochem Biophys Res Commun 2000; 269(1): 41-5.
[http://dx.doi.org/10.1006/bbrc.2000.2245] [PMID: 10694474]

[143] Huang Q, Rivest R, Richard D. Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. Endocrinology 1998; 139(4): 1524-32.
[http://dx.doi.org/10.1210/endo.139.4.5889] [PMID: 9528930]

[144] Darambazar G, Nakata M, Okada T, *et al.* Paraventricular NUCB2/nesfatin-1 is directly targeted by leptin and mediates its anorexigenic effect. Biochem Biophys Res Commun 2015; 456(4): 913-8.
[http://dx.doi.org/10.1016/j.bbrc.2014.12.065] [PMID: 25534851]

[145] Akieda-Asai S, Poleni PE, Date Y. Coinjection of CCK and leptin reduces food intake *via* increased CART/TRH and reduced AMPK phosphorylation in the hypothalamus. Am J Physiol Endocrinol Metab 2014; 306(11): E1284-91.
[http://dx.doi.org/10.1152/ajpendo.00664.2013] [PMID: 24735891]

[146] Liao GY, An JJ, Gharami K, *et al.* Dendritically targeted Bdnf mRNA is essential for energy balance and response to leptin. Nat Med 2012; 18(4): 564-71.
[http://dx.doi.org/10.1038/nm.2687] [PMID: 22426422]

[147] Yu S, Qualls-Creekmore E, Rezai-Zadeh K, *et al.* Glutamatergic Preoptic Area Neurons That Express Leptin Receptors Drive Temperature-Dependent Body Weight Homeostasis. J Neurosci 2016; 36(18): 5034-46.
[http://dx.doi.org/10.1523/JNEUROSCI.0213-16.2016] [PMID: 27147656]

[148] Hommel JD, Trinko R, Sears RM, *et al.* Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 2006; 51(6): 801-10.
[http://dx.doi.org/10.1016/j.neuron.2006.08.023] [PMID: 16982424]

[149] Xu L, Scheenen WJJM, Leshan RL, *et al.* Leptin signaling modulates the activity of urocortin 1 neurons in the mouse nonpreganglionic Edinger-Westphal nucleus. Endocrinology 2011; 152(3): 979-88.
[http://dx.doi.org/10.1210/en.2010-1143] [PMID: 21209012]

[150] Scott MM, Williams KW, Rossi J, Lee CE, Elmquist JK. Leptin receptor expression in hindbrain Glp-1 neurons regulates food intake and energy balance in mice. J Clin Invest 2011; 121(6): 2413-21.
[http://dx.doi.org/10.1172/JCI43703] [PMID: 21606595]

[151] Hayes MR, Skibicka KP, Leichner TM, *et al.* Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. Cell Metab 2010; 11(1): 77-83.
[http://dx.doi.org/10.1016/j.cmet.2009.10.009] [PMID: 20074530]

[152] Kim JG, Suyama S, Koch M, *et al.* Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and feeding. Nat Neurosci 2014; 17(7): 908-10.
[http://dx.doi.org/10.1038/nn.3725] [PMID: 24880214]

[153] White DW, Tartaglia LA. Leptin and OB-R: Body weight regulation by a cytokine receptor. Cytokine Growth Factor Rev 1996; 7(4): 303-9.
[http://dx.doi.org/10.1016/S1359-6101(96)00040-8] [PMID: 9023054]

[154] Tartaglia LA, Dembski M, Weng X, *et al.* Identification and expression cloning of a leptin receptor,

OB-R. Cell 1995; 83(7): 1263-71.
[http://dx.doi.org/10.1016/0092-8674(95)90151-5] [PMID: 8548812]

[155] Zhang J, Scarpace PJ. The soluble leptin receptor neutralizes leptin-mediated STAT3 signalling and anorexic responses *in vivo*. Br J Pharmacol 2009; 158(2): 475-82.
[http://dx.doi.org/10.1111/j.1476-5381.2009.00246.x] [PMID: 19422379]

[156] Hileman SM, Pierroz DD, Masuzaki H, *et al.* Characterizaton of short isoforms of the leptin receptor in rat cerebral microvessels and of brain uptake of leptin in mouse models of obesity. Endocrinology 2002; 143(3): 775-83.
[http://dx.doi.org/10.1210/endo.143.3.8669] [PMID: 11861497]

[157] Bjørbæk C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. J Biol Chem 1997; 272(51): 32686-95.
[http://dx.doi.org/10.1074/jbc.272.51.32686] [PMID: 9405487]

[158] Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 1979; 282(5738): 503-5.
[http://dx.doi.org/10.1038/282503a0] [PMID: 116135]

[159] McGowan MK, Andrews KM, Kelly J, Grossman SP. Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. Behav Neurosci 1990; 104(2): 373-85.
[http://dx.doi.org/10.1037/0735-7044.104.2.373] [PMID: 2189441]

[160] Air EL, Strowski MZ, Benoit SC, *et al.* Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nat Med 2002; 8(2): 179-83.
[http://dx.doi.org/10.1038/nm0202-179] [PMID: 11821903]

[161] Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci 2002; 5(6): 566-72.
[http://dx.doi.org/10.1038/nn0602-861] [PMID: 12021765]

[162] Brüning JC, Gautam D, Burks DJ, *et al.* Role of brain insulin receptor in control of body weight and reproduction. Science 2000; 289(5487): 2122-5.
[http://dx.doi.org/10.1126/science.289.5487.2122] [PMID: 11000114]

[163] Schwartz MW, Sipols AJ, Marks JL, *et al.* Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology 1992; 130(6): 3608-16.
[http://dx.doi.org/10.1210/endo.130.6.1597158] [PMID: 1597158]

[164] Sipols AJ, Baskin DG, Schwartz MW. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. Diabetes 1995; 44(2): 147-51.
[http://dx.doi.org/10.2337/diab.44.2.147] [PMID: 7859932]

[165] Benoit SC, Air EL, Coolen LM, *et al.* The catabolic action of insulin in the brain is mediated by melanocortins. J Neurosci 2002; 22(20): 9048-52.
[http://dx.doi.org/10.1523/JNEUROSCI.22-20-09048.2002] [PMID: 12388611]

[166] Polonsky KS, Given BD, Hirsch L, *et al.* Quantitative study of insulin secretion and clearance in normal and obese subjects. J Clin Invest 1988; 81(2): 435-41.
[http://dx.doi.org/10.1172/JCI113338] [PMID: 3276729]

[167] Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest 1988; 81(2): 442-8.
[http://dx.doi.org/10.1172/JCI113339] [PMID: 3276730]

[168] Figlewicz DP. Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. Am J Physiol Regul Integr Comp Physiol 2003; 284(4): R882-92.
[http://dx.doi.org/10.1152/ajpregu.00602.2002] [PMID: 12626355]

[169] Moller DE, Yokota A, Caro JF, Flier JS. Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol 1989; 3(8): 1263-9.
[http://dx.doi.org/10.1210/mend-3-8-1263] [PMID: 2779582]

[170] Szabo O, Szabo AJ. Evidence for an insulin-sensitive receptor in the central nervous system. Am J Physiol 1972; 223(6): 1349-53.
[http://dx.doi.org/10.1152/ajplegacy.1972.223.6.1349] [PMID: 4641627]

[171] Marks JL, Porte D Jr, Stahl WL, Baskin DG. Localization of insulin receptor mRNA in rat brain by *in situ* hybridization. Endocrinology 1990; 127(6): 3234-6.
[http://dx.doi.org/10.1210/endo-127-6-3234] [PMID: 2249648]

[172] Margolis RU, Altszuler N. Insulin in the cerebrospinal fluid. Nature 1967; 215(5108): 1375-6.
[http://dx.doi.org/10.1038/2151375a0] [PMID: 6055448]

[173] Young WS III. Periventricular hypothalamic cells in the rat brain contain insulin mRNA. Neuropeptides 1986; 8(2): 93-7.
[http://dx.doi.org/10.1016/0143-4179(86)90035-1] [PMID: 3531909]

[174] Devaskar SU, Singh BS, Carnaghi LR, Rajakumar PA, Giddings SJ. Insulin II gene expression in rat central nervous system. Regul Pept 1993; 48(1-2): 55-63.
[http://dx.doi.org/10.1016/0167-0115(93)90335-6] [PMID: 8265817]

[175] Devaskar SU, Giddings SJ, Rajakumar PA, Carnaghi LR, Menon RK, Zahm DS. Insulin gene expression and insulin synthesis in mammalian neuronal cells. J Biol Chem 1994; 269(11): 8445-54.
[http://dx.doi.org/10.1016/S0021-9258(17)37214-9] [PMID: 8132571]

[176] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005; 26(3): 439-51.
[http://dx.doi.org/10.1210/er.2005-0005] [PMID: 15897298]

[177] Tomas E, Tsao TS, Saha AK, *et al.* Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 2002; 99(25): 16309-13.
[http://dx.doi.org/10.1073/pnas.222657499] [PMID: 12456889]

[178] Yamauchi T, Kamon J, Waki H, *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001; 7(8): 941-6.
[http://dx.doi.org/10.1038/90984] [PMID: 11479627]

[179] Shklyaev S, Aslanidi G, Tennant M, *et al.* Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc Natl Acad Sci USA 2003; 100(24): 14217-22.
[http://dx.doi.org/10.1073/pnas.2333912100] [PMID: 14617771]

[180] Yamauchi T, Kamon J, Ito Y, *et al.* Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003; 423(6941): 762-9.
[http://dx.doi.org/10.1038/nature01705] [PMID: 12802337]

[181] Fry M, Smith PM, Hoyda TD, *et al.* Area postrema neurons are modulated by the adipocyte hormone adiponectin. J Neurosci 2006; 26(38): 9695-702.
[http://dx.doi.org/10.1523/JNEUROSCI.2014-06.2006] [PMID: 16988040]

[182] Guillod-Maximin E, Roy AF, Vacher CM, *et al.* Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. J Endocrinol 2009; 200(1): 93-105.
[http://dx.doi.org/10.1677/JOE-08-0348] [PMID: 18971219]

[183] Qi Y, Takahashi N, Hileman SM, *et al.* Adiponectin acts in the brain to decrease body weight. Nat Med 2004; 10(5): 524-9.
[http://dx.doi.org/10.1038/nm1029] [PMID: 15077108]

[184] Hoyda TD, Samson WK, Ferguson AV. Adiponectin depolarizes parvocellular paraventricular nucleus neurons controlling neuroendocrine and autonomic function. Endocrinology 2009; 150(2): 832-40.
[http://dx.doi.org/10.1210/en.2008-1179] [PMID: 18948398]

[185] De Rosa A, Monaco ML, Capasso M, *et al.* Adiponectin oligomers as potential indicators of adipose

tissue improvement in obese subjects. Eur J Endocrinol 2013; 169(1): 37-43.
[http://dx.doi.org/10.1530/EJE-12-1039] [PMID: 23612446]

[186] Meyer LK, Ciaraldi TP, Henry RR, Wittgrove AC, Phillips SA. Adipose tissue depot and cell size dependency of adiponectin synthesis and secretion in human obesity. Adipocyte 2013; 2(4): 217-26.
[http://dx.doi.org/10.4161/adip.24953] [PMID: 24052897]

[187] Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol 1994; 14(2): 1431-7.
[PMID: 8289818]

[188] Romacho T, Villalobos LA, Cercas E, Carraro R, Sánchez-Ferrer CF, Peiró C. Visfatin as a novel mediator released by inflamed human endothelial cells. PLoS One 2013; 8(10)e78283
[http://dx.doi.org/10.1371/journal.pone.0078283] [PMID: 24130902]

[189] Romacho T, Azcutia V, Vázquez-Bella M, *et al.* Extracellular PBEF/NAMPT/visfatin activates pro-inflammatory signalling in human vascular smooth muscle cells through nicotinamide phosphoribosyltransferase activity. Diabetologia 2009; 52(11): 2455-63.
[http://dx.doi.org/10.1007/s00125-009-1509-2] [PMID: 19727662]

[190] Tu TH, Nam-Goong IS, Lee J, Yang S, Kim JG. Visfatin Triggers Anorexia and Body Weight Loss through Regulating the Inflammatory Response in the Hypothalamic Microglia. Mediators Inflamm 2017; 2017: 1-8.
[http://dx.doi.org/10.1155/2017/1958947] [PMID: 29362519]

[191] Gao Q, Wolfgang MJ, Neschen S, *et al.* Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. Proc Natl Acad Sci USA 2004; 101(13): 4661-6.
[http://dx.doi.org/10.1073/pnas.0303992101] [PMID: 15070774]

[192] Bates SH, Stearns WH, Dundon TA, *et al.* STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 2003; 421(6925): 856-9.
[http://dx.doi.org/10.1038/nature01388] [PMID: 12594516]

[193] Kaelin CB, Gong L, Xu AW, *et al.* Signal transducer and activator of transcription (stat) binding sites but not stat3 are required for fasting-induced transcription of agouti-related protein messenger ribonucleic acid. Mol Endocrinol 2006; 20(10): 2591-602.
[http://dx.doi.org/10.1210/me.2006-0107] [PMID: 16709597]

[194] Xu AW, Ste-Marie L, Kaelin CB, Barsh GS. Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (Pomc) neurons causes decreased pomc expression, mild obesity, and defects in compensatory refeeding. Endocrinology 2007; 148(1): 72-80.
[http://dx.doi.org/10.1210/en.2006-1119] [PMID: 17023536]

[195] Buettner C, Pocai A, Muse ED, Etgen AM, Myers MG Jr, Rossetti L. Critical role of STAT3 in leptin's metabolic actions. Cell Metab 2006; 4(1): 49-60.
[http://dx.doi.org/10.1016/j.cmet.2006.04.014] [PMID: 16814732]

[196] Thon M, Hosoi T, Ozawa K. Insulin enhanced leptin-induced STAT3 signaling by inducing GRP78. Sci Rep 2016; 6(1): 34312.
[http://dx.doi.org/10.1038/srep34312] [PMID: 27677243]

[197] Coope A, Milanski M, Araújo EP, *et al.* AdipoR1 mediates the anorexigenic and insulin/leptin-like actions of adiponectin in the hypothalamus. FEBS Lett 2008; 582(10): 1471-6.
[http://dx.doi.org/10.1016/j.febslet.2008.03.037] [PMID: 18394428]

[198] Kim JY, Bae YH, Bae MK, *et al.* Visfatin through STAT3 activation enhances IL-6 expression that promotes endothelial angiogenesis. Biochim Biophys Acta Mol Cell Res 2009; 1793(11): 1759-67.
[http://dx.doi.org/10.1016/j.bbamcr.2009.09.006] [PMID: 19751774]

[199] Rizk NM, Stammsen D, Preibisch G, Eckel J. Leptin and tumor necrosis factor-alpha induce the tyrosine phosphorylation of signal transducer and activator of transcription proteins in the

hypothalamus of normal rats *in vivo*. Endocrinology 2001; 142(7): 3027-32.
[http://dx.doi.org/10.1210/endo.142.7.8225] [PMID: 11416024]

[200] Lambert PD, Anderson KD, Sleeman MW, *et al.* Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. Proc Natl Acad Sci USA 2001; 98(8): 4652-7.
[http://dx.doi.org/10.1073/pnas.061034298] [PMID: 11259650]

[201] Buchanan JB, Johnson RW. Regulation of food intake by inflammatory cytokines in the brain. Neuroendocrinology 2007; 86(3): 183-90.
[http://dx.doi.org/10.1159/000108280] [PMID: 17823502]

[202] Auernhammer CJ, Bousquet C, Melmed S. Autoregulation of pituitary corticotroph SOCS-3 expression: Characterization of the murine SOCS-3 promoter. Proc Natl Acad Sci USA 1999; 96(12): 6964-9.
[http://dx.doi.org/10.1073/pnas.96.12.6964] [PMID: 10359822]

[203] Yang G, Lim CY, Li C, *et al.* FoxO1 inhibits leptin regulation of pro-opiomelanocortin promoter activity by blocking STAT3 interaction with specificity protein 1. J Biol Chem 2009; 284(6): 3719-27.
[http://dx.doi.org/10.1074/jbc.M804965200] [PMID: 19049975]

[204] Harris M, Aschkenasi C, Elias CF, *et al.* Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. J Clin Invest 2001; 107(1): 111-20.
[http://dx.doi.org/10.1172/JCI10741] [PMID: 11134186]

[205] Mütze J, Roth J, Gerstberger R, Hübschle T. Nuclear translocation of the transcription factor STAT5 in the rat brain after systemic leptin administration. Neurosci Lett 2007; 417(3): 286-91.
[http://dx.doi.org/10.1016/j.neulet.2007.02.074] [PMID: 17353091]

[206] Rahmouni K, Sigmund CD, Haynes WG, Mark AL. Hypothalamic ERK mediates the anorectic and thermogenic sympathetic effects of leptin. Diabetes 2009; 58(3): 536-42.
[http://dx.doi.org/10.2337/db08-0822] [PMID: 19066310]

[207] Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG Jr, Schwartz MW. Key enzyme in leptin-induced anorexia. Nature 2001; 413(6858): 794-5.
[http://dx.doi.org/10.1038/35101657] [PMID: 11677594]

[208] Rogers PJ, Smit HJ. Food craving and food "addiction": a critical review of the evidence from a biopsychosocial perspective. Pharmacol Biochem Behav 2000; 66(1): 3-14.
[http://dx.doi.org/10.1016/S0091-3057(00)00197-0] [PMID: 10837838]

[209] Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci 2005; 8(11): 1445-9.
[http://dx.doi.org/10.1038/nn1578] [PMID: 16251986]

[210] Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2001; 2(2): 119-28.
[http://dx.doi.org/10.1038/35053570] [PMID: 11252991]

[211] Rada P, Avena NM, Hoebel BG. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 2005; 134(3): 737-44.
[http://dx.doi.org/10.1016/j.neuroscience.2005.04.043] [PMID: 15987666]

[212] Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci 2011; 15(1): 37-46.
[http://dx.doi.org/10.1016/j.tics.2010.11.001] [PMID: 21109477]

[213] Boutrel B, Kenny PJ, Specio SE, *et al.* Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. Proc Natl Acad Sci USA 2005; 102(52): 19168-73.
[http://dx.doi.org/10.1073/pnas.0507480102] [PMID: 16357203]

[214] Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. Nature 2005; 437(7058): 556-9.
[http://dx.doi.org/10.1038/nature04071] [PMID: 16100511]

[215] Barrot M, Olivier JDA, Perrotti LI, *et al.* CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc Natl Acad Sci USA 2002; 99(17): 11435-40. [http://dx.doi.org/10.1073/pnas.172091899] [PMID: 12165570]

[216] Olausson P, Jentsch JD, Tronson N, Neve RL, Nestler EJ, Taylor JR. DeltaFosB in the nucleus accumbens regulates food-reinforced instrumental behavior and motivation. J Neurosci 2006; 26(36): 9196-204. [http://dx.doi.org/10.1523/JNEUROSCI.1124-06.2006] [PMID: 16957076]

# CHAPTER 6

# Body Fluid Homeostasis and Water Intake Regulation

**Abstract:** Water is essential for life and a major key for survival. It is originated mostly from drinking, feedstuffs, and metabolic pathways (metabolic water). Water comprises from 75% body weight in infants to 55% in the elderly and is essential for cellular homeostasis. Thirst and water consumption are centrally and peripherally regulated by complex and tightly interconnected mechanisms. This chapter summarizes current progress and knowledge associated with body fluid homeostasis and the regulation of water intake at the central and peripheral levels.

**Keywords:** Central circuit, ncRNA, Osomoregulation, Peripheral pathways, RAAS, Thirst, TRPV, Water intake.

## INTRODUCTION

As I stated in Chapter 1, we drink because we are thirsty. Water is the most abundant constituent (50-60% of body weight) in the body. Approximately 55-75% of total body water is in the intracellular compartment, and the rest (~25-45%) in the extracellular compartment with a ratio of 1:3 intravascular (plasma) and extravascular (interstitial) spaces. Animals and humans continuously lose water by various physiological and cellular processes, including sweating, urination, and basal metabolic activity. To maintain water homeostasis and compensate for such losses, animals must drink sufficient water and ingest food from external sources. The maintenance of this in-and-out water balance represents a key homeostatic function for survival in all organisms. It specifically occurs through a balance between water intake/excretion and salt intake/excretion to keep the osmolality of the extracellular fluid at the optimal set-point. These processes are finely and tightly controlled at the entire organism level, including the peripheral sensory system and the central neural circuits. This chapter highlights recent advances in the field and describes the molecular mechanisms involved in the regulation of body fluid homeostasis.

**Sami Dridi**

## 6.1. Organs Involved in Osmoregulation

Based on the Encyclopaedia, osmoregulation is the maintenance by an organism of an internal balance between water and dissolved materials regardless of environmental conditions. As water and sodium are associated (where goes sodium, water soon follows whether by osmosis[1] or bolus flows), all organisms have to regulate sodium and water in order to remain in homeostasis. Several organs, depending on vertebrate species, are involved in osmoregulation to inhabit a wide variety of environments. With the exception of the mammals where the only kidney is involved, all other vertebrates use more than one organ/system to maintain the osmoregulatory homeostasis. Birds, for instance, use kidney, intestine, and salt glands for the maintenance of fluid and electrolyte balance. Reptiles utilize the kidney, intestine, bladder, and salt glands. Fish employ kidneys, intestine, bladder, and gills. In addition to the skin, amphibians make use of the same organs as fish. It is noteworthy that mammals can lose water and electrolytes *via* various routes, including skin, lungs, GI, but not for osmoregulation. The mammalian urinary bladder is a urine storage organ and is not involved in osmoregulation [1].

Changes in body water/sodium balance disturb the extracellular fluid volume, which in turn affects arterial blood pressure. The CNS receives continuous inputs from the peripheral organs about the status of ECF osmolality, sodium concentration, sense of taste, fluid volume, and blood pressure, and acts accordingly to adjust the body fluid homeostasis.

## 6.2. Water and Sodium Taste

The question that one might ask is whether water has a taste receptor or not. In Drosophila, water taste is mediated by ppk28, a member of the epithelial sodium channel/degenerin (ENac/Deg) family, which is expressed in gustatory receptor neurons [2, 3]. In fact, functional studies showed that water consumption was reduced in flies lacking ppk28 [4]. In mammals, however, data are not conclusive, although electrophysiological studies have shown that water can stimulate taste nerves in several species, including cats and dogs [5]. Recent study showed that water and sour (acid) tastes are encoded by the same taste receptor cells [6]. Zocchi *et al*. [6] demonstrated that the application of water on the tongue selectively activates PKD2L1, a member of the taste receptor cell channel family.

Sodium (salt), depending on its concentration, can result in two opposite behaviors: appetitive at low to moderate concentration and aversive at high levels [7]. These two-opposing behavioral responses are mediated by distinct anatomical and molecular pathways. It has been shown that knocking out the epithelial

sodium channel (ENaC) alpha subunit abolished attraction and taste nerve response to low salt without affecting aversive response-induced by high salt [8, 9]. High salt, however, recruits additional pathways such as bitter- and sour-sensing taste receptor cells.

## 6.3. Central Sensing Mechanisms for Internal Water Homeostasis and Thirst Regulation

### *6.3.1. Neurochemical Circuits*

Thirst is defined as the conscious need for water or liquid, and its onset starts when the osmolality of the blood rises above a threshold of ~292 mOsm/kg water, which in turn triggers vasopressin increase, water intake, and varying renal urine flow [10]. Thus, vasopressin secretion, water ingestion, and the renal concentrating mechanism work jointly to maintain body fluid osmolality and water set-point. For instance, high extracellular fluid (ECF) osmolality stimulates the thirst sensation, promotes water intake, increases vasopressin release, and enhances water reabsorption in the kidney [11]. Water deprivation or salt ingestion/administration increases plasma osmolality however drinking water lowers osmolality in dehydrated individuals [12, 13].

Peripheral and central osmoreceptors are postulated to sense the rapid change in local osmolality and exchange information that leads to behavioral modulation and maintenance of water homeostasis. Osmoreceptors are defined as neurons that are endowed with an intrinsic ability to detect changes in ECF osmolality [14]. The presence of central osmoreceptors is known 80 years ago when Verney E.B. correlated the effects of carotid infusion of various osmolytes on urine output and the release of the antidiuretic hormone [15]. The primary central and dominant "osmostat" is the lamina terminalis (LT), which is located in the forebrain and is the main brain structure monitoring internal water balance by detecting blood tonicity and sensing blood osmolality [14]. This brain region contains the subfornical organ (SFO), the organum vasculosum of LT (OVLT), and the median preoptic nucleus (MnPO) (Fig. **6.1**). Furthermore, injection of hypertonic solutions in this brain area provokes thirst and vasopressin release [16]. Experimental ablation of the OVLT and adjacent regions leads to a defect in water intake and VP release [17]. Combined ablation of all three regions; OVLT, SFO, and MnPO is required for complete abolition of thirst and osmotic-induced VP release [18]. The osmoreceptive and osmosensitive neurons are largely distributed within the CNS, including OVLT, MnPO, and SFO [19]. Neurons from the OVLT and SFO project to magnocellular neurons within the supraoptic (SON) and paraventricular (PVN) nuclei [20] (Fig. **6.1**). These neurons are activated by

hypertonic conditions. Signals detected by peripheral organs and osmoreceptors (taste receptors, peripheral osmo-sodium, volume receptors, and arterial/cardiopulmonary baroreceptors) reach the CNS through the cranial nerves to the nucleus of the solitary tract (NTS) or *via* the OVLT/SFO that contain cells sensitive to humoral signals associated with sodium concentration changes in plasma and CSF, osmolality, and angiotensin II (ANGII) levels [21, 22]. These humoral and neural signals activate central circuits that include the MnPO, PVN, SON, lateral parabrachial nucleus (LPBN), dorsal raphe nucleus (DRN), and neurochemical systems such as angiotensinergic, vasopressinergic, oxytocinergic, and serotonergic systems to trigger appropriate behavioral, endocrine, and sympathetic responses and adjust body fluid homeostasis. For instance, it has been shown that ANGII induced drinking *via* its receptor Agtr1a, which is highly enriched in the LT [23].

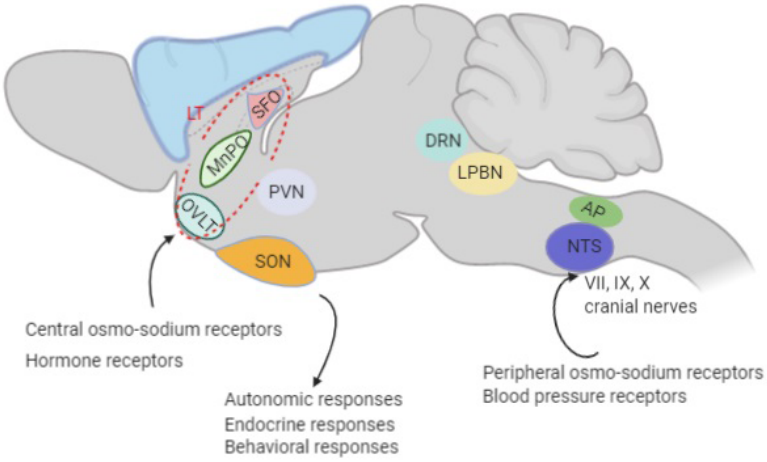


**Fig. (6.1). Central neurochemical circuits are involved in the regulation of body fluid homeostasis.** DRN, dorsal raphe nucleus; LPBN, lateral parabrachial nucleus; LT, lamina terminalis; MnOP, median preoptic nucleus; OVLT, organum vasculosum of LT; PVN, paraventricular nucleus; SFO, subfornical organ; SON, supraoptic nucleus. The figure was made using BioRender.

## 6.4. Peripheral Osmoreceptors

Studies from rodents to primates and humans indicated that there are peripheral osmoreceptors. Kuramoch and Kobayashi [24] have shown that such receptors are located in the oropharyngeal cavity. The GIT [13, 25], the hepatic portal vein [26], the liver [27], and the splanchnic mesentery [28] have been shown to also site that harbor osmoreceptors. Although the cellular and molecular structures of the peripheral osmoreceptors are not well defined, it has been shown that the information they collect reaches the CNS *via* the vagus nerve [29, 30]. In fact,

nodose ganglion osmosensitive neurons from which axons that ascend in the vagus nerve can make synapses in the NTS [14] (Fig. **6.2**).

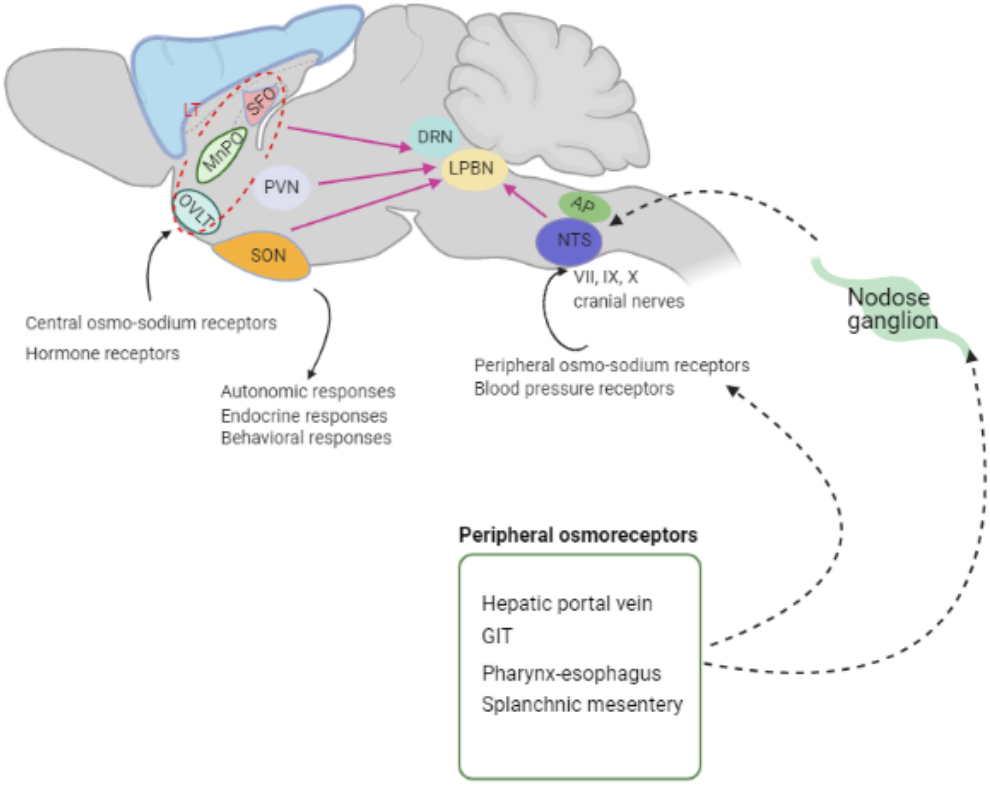


**Fig. (6.2). Peripheral osmoregulatory circuits.** Schematics showing that the NTS and LPBN integrate peripheral and visceral signals and communicate with LT area to regulate body fluid homeostasis. GIT, gastrointestinal tract; DRN, dorsal raphe nucleus; LPBN, lateral parabrachial nucleus; LT, lamina terminalis; MnOP, median preoptic nucleus; OVLT, organum vasculosum of LT; PVN, paraventricular nucleus; SFO, subfornical organ; SON, supraoptic nucleus. The figure was made using BioRender.

## 6.5. Molecular Basis of Body Fluid Regulation

### *6.5.1. Transient Receptor Potential Vanilloid (TRPV) Channels*

Members of the TRP cation channels have been implicated in neuronal osmosensing since many subtypes are blocked by gadolinium and ruthenium red, potent inhibitors of osmosensory transduction in OVLT [31, 32]. The mammalian TRPV type 4 (TRPV4) was first identified by Liedtke and co-workers [33] and found to be expressed in osmoreceptor neurons in the OVLT and MnPO. TRPV4 knockout mice manifested reduced drinking behavior, which was associated with

a mild increase in serum osmolality [34]. After water deprivation or intraperitoneal administration of hypertonic saline solution, the $TRPV^{-/-}$ mice had increased serum osmolality, a blunted increase in vasopressin, and attenuated induction of induction OVLT c-fos expression [34]. Furthermore, ICV infusion of a TRPV4 agonist reduces both spontaneous and ANGII-induced drinking, but not that of water deprivation-induced drinking [35]. In contrast to $TRPV4^{-/-}$ mice developed by Liedtke *et al*., Mizuno and colleagues found in a separate TRPV4 KO mice no difference in water intake and serum osmolality, however they noticed higher vasopressin secretion [36].

Similar to TRPV4, TRPV1 has also been implicated in the activation of osmoreceptor neurons by hypertonic stimuli. First, TRPV1 is expressed in SON, OVLT, and vasopressin neurons [31, 37]. $TRPV1^{-/-}$ mice exhibit an impairment of osmotic-induced vasopressin release [37]. When intraperitoneally challenged with hypertonic saline solution, $TRPV1^{-/-}$ showed a reduction in drinking water compared to control wild-type counterparts [31]. In another study conducted by Taylor and colleagues, $TRPV^{-/-}$ mice have no abnormality in water consumption induced by hypovolemic or osmotic stimuli, with no detectable difference in OVLT c-fos induction by hypertonicity [38].

TRPV2 has been shown to be co-localized in vasopressin and oxytocin magnocellular neurons of the SON and PVN in an animal model of hyponatremia[2] [39]. In primates, TRPV2 was found to be highly expressed in the hypothalamic paraventricular, suprachiasmatic, and supraoptic nucleus and colocalized with oxytocinergic and vasopressinergic neurons [40]. In rodents, TRPV2 is expressed in the SON, PVN, OVLT, LT, SOF, and MnPO, suggesting a potential role in the regulation of body fluid homeostasis [41].

### *6.5.2. The Renin-Angiotensin-Aldosterone System (RAAS)*

The RAAS is a peptidergic hormone system involved in the regulation of water and body fluid homeostasis and blood pressure. It is mainly comprised of the three hormones renin, angiotensin II (ANG II), and aldosterone. The first stage of the RAAS is the release of renin, which is produced from prorenin in the granular cells (juxtaglomerular, JG) of the kidney, in response to reduced sodium delivery to the distal convoluted tubule or reduced perfusion pressure in the kidney detected by baroreceptors [42]. Renin release is inhibited by increased blood pressure and the activation of the atrial natriuretic peptide (ANP).

Once it has been released into the circulation, Renin cleaves angiotensinogen, which is produced in the liver, to form Angiotensin I (ANGI). ANGI is further cleaved by the angiotensin converting enzyme (ACE) to produce the active

peptide ANGII. ANGII stimulates aldosterone release in the adrenal cortex, stimulates $Na^+$ and $Cl^-$ reabsorption in the kidney, and increases the thirst sensation and salt appetite in the hypothalamus. Peripheral or ICV administration of ANGII induced thirst-related behavior and caused a dose-response increase in water intake in various species [43]. Second, ANGII stimulates the release of antidiuretic hormone (ADH), also known as arginine vasopressin, by the posterior pituitary, which in turn increases fluid retention in the kidney *via* aquaporins and help conserve blood volume [44]. Hypovolemic treatments and dehydration increased circulating ANGII levels, which is associated with an increase in serum osmolality and $Na^+$ concentration. Activation of ANGII receptors in the brain also increases sympathetic output to the heart and vasculature, which increases cardiac output and total peripheral resistance, thus increasing blood pressure (Fig. **6.3**).

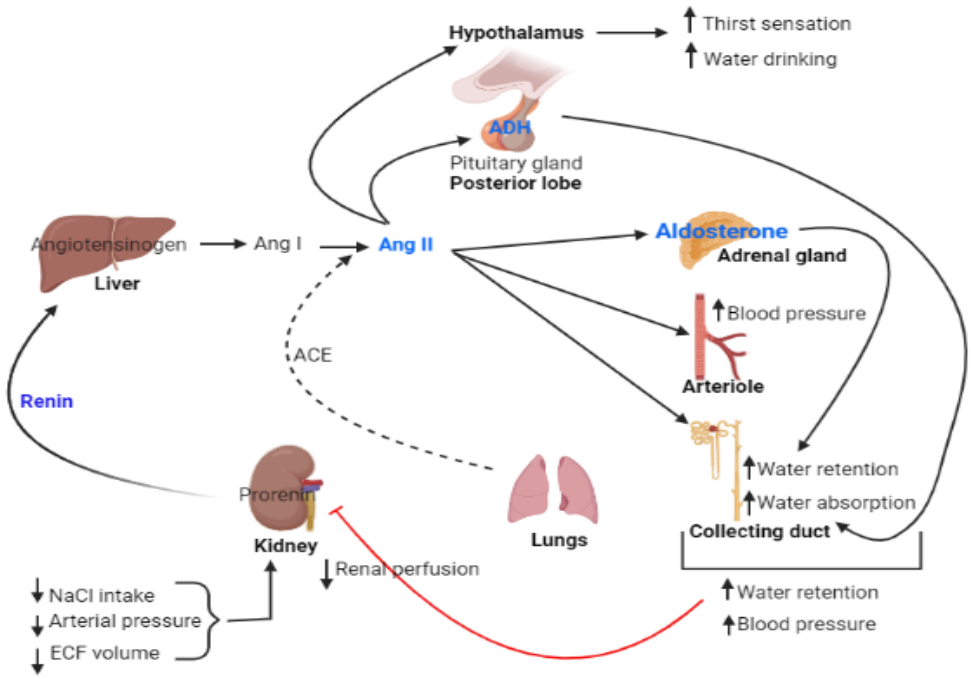


**Fig. (6.3). Role of RAAS in body fluid balance.** Renin catalyses the conversion of angiotensinogen into Ang I, which is converted by the ACE into Ang II. ANG II controls the secretion of aldosterone, which stimulates Na+ retention and ADH that stimulates H2O reabsorption by the kidney. Plasma volume and plasma osmolality control salt appetite and drinking behavior. High-Na+ intake leads to change in plasma volume and osmolality, which negatively affects renin secretion. ACE, angiotensin-converting enzyme; ADH, antidiuretic hormone; Ang I/II, angiotensin I/II; ECF, extracelluar fluid. The figure was made using BioRender.

It has been postulated that there are two RAAS, one peripheral and one central [45]. Indeed, ANGII modulates thirst sensation *via* two mechanisms:

1. The circulating ANGII may act on its receptor AT1 in the circumventricular organ (CVO) of the brain,
2. Central ANGII may act as a neurotransmitter, which coordinates and integrate osmotic and hormonal information.

ANGII exerts its action by binding to different receptors such as AT1 and AT2 that are widely distributed throughout the body, and many of its classical functions (aldosterone release and thirst induction) are mediated by AT1. This receptor subtype is predominantly coupled to the G protein Gq/11, and signals through phospholipases A, C, and D, inositol phosphates, calcium channels, and a variety of serine/threonine and tyrosine kinases.

### *6.5.3. Antidiuretic Hormone (ADH)*

ADH, also known as arginine vasopressin (AVP) or vasopressin, is a nonapeptide derived from the preprohormone prepropressophysin, and is mainly synthesized in the hypothalamic supraoptic nucleus, and to a less extent in the hypothalamic PVN, and released in the posterior pituitary to enter the body's systemic circulation [46]. ADH release is stimulated by hypovolemia[3] or hypernatremia[4] and it plays essential roles in the control of the body's osmotic balance, blood pressure regulation, sodium homeostasis, and kidney functioning.

As ADH is stored in the neurons that contain osmoreceptors and are responsive to blood osmolarity, elevation in osmolarity results in ADH secretion which primarily targets the kidney to increase water reabsorption [47]. During hypovolemia, baroreceptors sense low blood pressure and send information to the vagus nerve, which in turn stimulates ADH to release and promote water reabsorption in the kidney and induces vasoconstriction to increase blood volume and pressure [47]. It has been shown that central administration of ADH into the hypothalamic lateral septal area (LSA) increased water intake in a dose-dependent manner, and this effect was inhibited by central administration of ADH receptor (V1) antagonist into the PVN [48]. Three different receptors for ADH, V1aR, V1bR, also called V3R, and V2R, have been characterized [49]. The effects *via* V2R activation are mediated by cyclic AMP. For instance, ADH binds V2R in the principal kidney cells of the collecting duct and activates adenylate cyclase, which causes a subsequent increase in the second messenger cAMP, leading to the activation of the second messenger cAMP protein kinase A (PKA). PKA activation initiates an intracellular phosphorylation cascade, including

phosphorylation of the water channel aquaporin 2 (AQP2), which moves into the luminal membrane of the collecting duct cells and increases water reabsorption [50, 51]. Additionally, *via* V2R and its action on the luminal sodium channel ENac, ADH stimulates sodium reabsorption in the cortical and outer medullary collecting tubes, which helps concentrate all solutes in the lumen. In the terminal inner medullary collecting tubes and *via* urea transporters UT-A1 and UT-A3, ADH increases the urea concentration favoring water reabsorption [52].

The effects of ADH following V1aR and V1bR activation are mediated by calcium signals. For example, in vascular smooth muscle, ADH activates G protein, which phosphorylates phospholipase C (PLC) and produces inositol triphosphate (IP-3) and diacylglycerol (DAG), leading to increased release of intracellular calcium from the endoplasmic reticulum. Calcium and DAG activate protein kinase C (PKC) and its downstream phosphorylation cascades leading to a contraction of vascular smooth muscle and increased blood pressure (Fig. **6.4**).

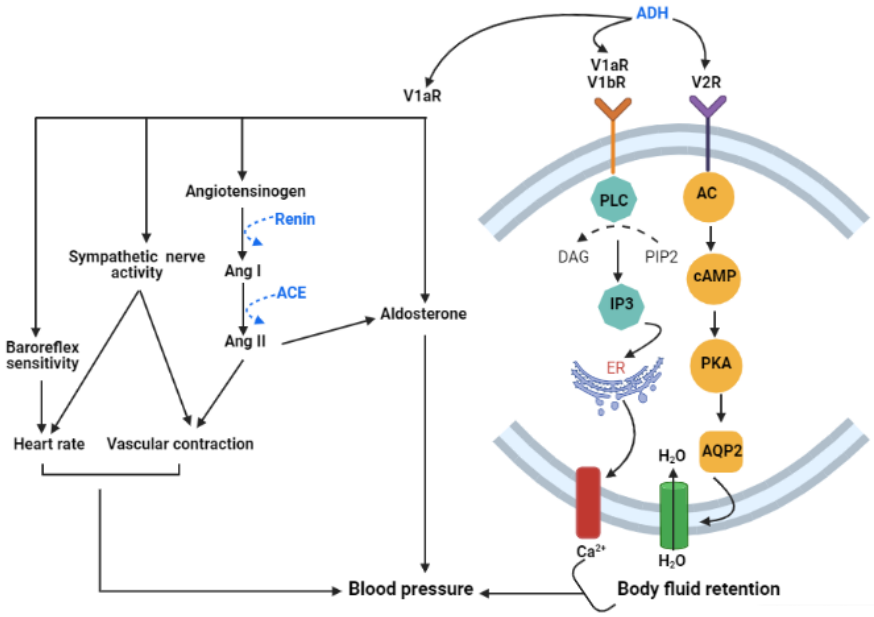


**Fig. (6.4). Schematic illustration of ADH system contribution in the regulation of body fluid and blood pressure homeostasis.** ADH binds to V2R, activates AC-cAMP-PKA pathway, and induces the translocation of AQP2 and water reabsorption in the kidney. Similarly, the ADH/V1aR system activates PLC pathways and $Ca^{2+}$ signaling. Both systems regulate blood pressure. ADH/V1aR also stimulates RAS activity and aldosterone release. In addition, AVP stimulates vascular contraction through V1aR and enhances baroreflex sensitivity, which controls the heart rate. All together regulate blood pressure. AC, adenylate cyclase; ACE, angiotensin converting enzyme; ADH, Antidiuretic hormone; Ang I/II, angiotensin I/II; AQP2, aquaporin 2; DAG, diacylglycerol; IP3, inositol triphosphate.

### *6.5.4. Oxytocin*

Oxytocin is a 9 amino-acid peptide hormone closely related to ADH with seven identical amino acids and is both released from the posterior pituitary. Depending on species and hormone dosage, oxytocin has been found to have both diuretic and antidiuretic functions. Early studies showed that peripheral administration of oxytocin raised the urinary excretion of sodium [53]. Li *et al*. [54] reported that oxytocin infusion increased urine osmolality and solute-free water reabsorption in Brattleboro rats. Bernal and co-workers showed that subcutaneous administration of oxytocin increased water intake and urine excretion in food-deprived rodents [55].

As receptors for oxytocin and ADH share structural homology and are G protein-coupled receptors, oxytocin can bind to V2R with low affinity [56], which may explain its antidiuretic activity. Using inner medullary collecting duct (IMCD) cells, Jeon *et al*. [57] showed that oxytocin induce AQP redistribution in predominantly apical and subapical localization. Furthermore, peripheral administration of oxytocin induces AQP2 translocation into the apical plasma membrane [54]. Together, these studies support the role of oxytocin in stimulating AQP2 trafficking in the kidney and indicating a similar role as ADH.

### *6.5.5. Secretin*

Secretin is a peptide hormone encoded by the SCT gene and mainly produced in the S cells of the duodenum. Its primary function is to modulate water and electrolyte transport in pancreatic duct cells [58], liver cholangiocytes [59], and epididymis epithelial cells [60]. Earlier studies suggested a diuretic function of secretin in humans and dogs [61]. In fact, secretin administration caused a rise in urinary water, sodium, calcium, and solute excretion [62]. Transgenic secretin receptor (SCTR)-null mice drank more water and exhibited polyuria[5] and polydipsia[6] phenotype compared to their wild-type counterparts [63]. These mice also showed reduced levels of AQP2 and AQP4 in the kidney [63]. *In vitro* study using inner medullary tubular cells showed that secretin increased AQP2 levels in a dose-dependent manner. Consistent with this, Li *et al*. (2001) showed that secretin induces AQP2 redistribution from the intracellular vesicles to the plasma membrane in inner medullary tubular cells from rat kidneys. This effect was abolished by the secretin antagonist and cAMP-PKA inhibitor.

Immunohistochemical staining demonstrated the presence of SCTR in the cuboidal epithelium of the collecting ducts and the proximal tubules and the thick ascending segment of the loop of Henle [63].

Secretin was also found in the magnocellular neurons of the PVN and supraoptic nucleus and along the neurohypophysial tract. The role of secretin in water regulation was further supported by the observation that secretin levels increased during water deprivation [64]. As the expression of V2R did not differ between the $SCTR^{-/-}$ and their wild-type counterparts, the data suggest that secretin regulates water homeostasis independently of ADH. ICV administration of secretin has been shown to increase water intake in coordination with ANGII and oxytocin [65, 66]. Additional pieces of evidence came from the co-localization of secretin and AT1R in the PVN and the induction of secretin expression in SFO and OVLT following central injection of ANGII [66]. Similar to ADH, secretin increases intracellular cAMP levels *via* binding to SCTR, which is coupled to AC, leading to activation of PKA and AQP2 phosphorylation [67].

### *6.5.6. Serotonin*

Serotonin (also known as 5-hydroxytryptamine, 5-HT) was first identified in the CNS in 1953 [68]. Serotonin-containing neurons in the median raphe nucleus (MRN), dorsal raphe nucleus (DRN), and raphe centralis superior (B7–B9 groups) provide extensive serotonergic innervations to telencephalon and diencephalon, whereas the intermediate and posterior groups (B1–B6 groups) send local projections at the pons, and descendent projections to the mesencephalon, medulla and spinal cord [69]. Lesions of the DRN and MRN increased water intake in rodents [70, 71]. Peripheral administration of serotonin or its agonist, however, stimulates water intake [72, 73]. Pharmacological manipulation (activation/inhibition) of serotonin receptors also affects water intake. For instance, activation of central $5\text{-HT}_{1D}$ receptor following $_{L}$-694,247 administration decreases water intake in both dehydrated rodents and in rodents receiving a central injection of ANGII [74]. Similarly, injection of 8-OH-DPAT (8-hydrox--2-(di-n-propylamino)tetralin), a 5-HT1A agonist, into the PVN and LSA decreases water intake in water-deprived animals [75, 76]. Central administration of 5-HT2 agonists inhibits water intake in rodents [77, 78]. Interestingly, 5-HT4 receptor seemed to have dual effects depending on the physiological status of the animal. In fact, central administration of 5-HT4 antagonists stimulate water intake in hypovolemic animals but reduces water intake in hyperosmotic animals [79]. Together, these studies indicate that central 5-HT receptors inhibit water intake *via* angiotensinergic and cholinergic pathways. As for the water intake, central serotonergic receptors also control sodium appetite. Indeed, Castro *et al*. [80] showed that ICV administration of serotonergic agents reduces the salt intake in sodium-depleted rodents. Neil and Cooper [81] and Rouah-Rosilio *et al*. [82] have shown that modulation of the serotonergic system inhibits salt appetite and intake in rodents. *Via* its receptors, serotonin regulates blood volume, cardiac output, and blood pressure [83].

### *6.5.7. Aquaporins (AQPs)*

AQPs constitute a group of integral membrane proteins characterized by six transmembrane helices connected by five loops that are organized in monomers, dimers, and tetramers, forming pores in the cell membrane [84]. AQP0 was first characterized as a major intrinsic polypeptide (MIP) in the lens [85], followed by the isolation and identification of AQP1 in human erythrocytes and renal proximal tubule membranes by Denker *et al*. [86] and Preston and Agre [87]. Since these discoveries, several other AQPs (13 in total, AQP0-AQP12) were described and found to be widely distributed in the body (Table **6.1**). AQPs are subdivided into three classes including AQPs (AQP0, 1, 2, 4, 5, 6, 8) transporting water exclusively, aquaglyceroporins (AQP3, 7, 9, 10) transporting not only water but also glycerol, and superaquaporins or unorthodox AQPs (AQP11, 12) with a non-well defined function. To cite few examples, AQP2 for instance is expressed in collecting principal duct cells and is translocated into the apical membrane by exocytosis from a subapical pool of vesicles during ADH stimulation to induce water reabsorption and urine concentration [50]. Dysfunction of AQP2 in humans is associated with diabetes insipidus. AQP1 is expressed in the apical and basolateral membrane of kidney proximal tubule cells, thin descending limb cells and the endothelium of vasa recta. AQP1 knockout mice displayed lower urine osmolality compared to their wild-type counterparts [88]. AQP3 null mice manifested lower urine osmolality alongside higher urine volume and water intake, although they appeared grossly normal [89]. AQP4 null mice, however, had a normal growth but they were enable to concentrate urine during long period of water deprivation [90].

**Table 6.1. Aquaporins distribution and functions.**

| AQPs | Tissue Distribution And Cellular Localization | Physiological Function |
|---|---|---|
| AQP0 | Eye: lens fiber cells<br>Red blood cells | Fluid balance within the lens<br>Osmoregulation |
| AQP1 | Kidney: proximal tubule<br>Eye: ciliary epithelium<br>Brain: choroid plexus<br>Lung: alveolar epithelial cells | Urine concentration<br>Production of aqueous humor<br>Production of CSF[7]<br>Alveolar hydration |
| AQP2 | Kidney: collecting ducts | Mediate ADH[8] activity |
| AQP3 | Kidney: collecting ducts<br>Keratinocytes (epidermal)<br>Trachea: epithelial cells | Water reabsorption<br>Hydration and epidermal proliferation<br>Water secretion into the trachea |

*(Table 1) cont.....*

| AQPs | Tissue Distribution And Cellular Localization | Physiological Function |
|---|---|---|
| AQO4 | Kidney: collecting ducts<br>Brain: ependymal cells<br>Brain: hypothalamus<br>Brain: astrocytes<br>Lung: bronchial epithelium | Water reabsorption<br>CSF fluid balance<br>Osmosensing function<br>Water permeability<br>Bronchial fluid secretion |
| AQP5 | Salivary gland<br>Lacrimal glands | Saliva production<br>Tear production |
| AQP6 | Kidney | Water permeability |
| AQP7 | Adipocyte cells | Transport of glycerol out of adipocytes |
| AQP8 | Colon<br>liver | Colonic water adsorption<br>Hepatocyte bile formation |
| AQP9 | Brain | Transports energy substrates |
| AQP10 | Epithelial of organs | Permeate neutral solute (urea) |
| AQP11 | Brain, kidney, heart, ER | Role not clear yet |
| AQP12 | Pancreatic acinar cells | Secretion of digestive enzymes and fluid |

Although AQPs are well known to play key roles in water fluid homeostasis, recent advances showed that they are also involved in many other physiological functions, such as cell migration and inflammatory responses. This part is not in the scoop of this ebook, however, readers can refer to elegant reviews elsewhere [91].

### *6.5.8. Other Regulators of Water Fluid Homeostasis*

Several other molecular signatures involved in water fluid balance have been identified, such as NHE3, NKCC2, ENaC, NCC, CLC-K1, and ROMK. The sodium-hydrogen antiporter 3, also known as sodium-hydrogen exchanger 3 (NHE3), is a solute carrier family 9 members 3 and is expressed in the apical membrane of the proximal tubule and thick ascending limb. NHE3-null mice exhibited lower arterial blood pressure, decreased proximal reabsorption rate, and higher plasma $K^+$ compared to wild-type mice [92]. NHE3 knockout mice display a decreased protein expression of AQP2 in the inner medulla and cortex [93]. The Na-K-2Cl cotransporter (NKCC2) is located in the apical membrane of the epithelial cells of the thick ascending limb of the loop of Henle, and has higher reabsorptive activity in the kidney. It has been shown that changes in NKCC2 transport activity alter the renal reabsorptive capacity for NaCl and eventually lead to perturbations of the salt and water homeostasis [94]. Takahashi *et al*. [95] showed that NKCC2 knockout mice suffer severe dehydration and growth retardation and do not survive longer than 2 weeks after birth. The apical

epithelial sodium channel (ENaC) is composed of three homologous subunits (α, β, and γ) and allows the flow of $Na^+$ ions across high resistance epithelia, maintaining body salt and water homeostasis [96]. ENaCβ- and ENaCγ-KO mice exhibited a severe salt wasting phenotype and did not survive beyond 48h after birth [97]. Specific collecting duct ENaCα-KO mice had high plasma aldosterone concentrations [98]. The sodium-chloride cotransporter (NCC) is expressed in the distal convoluted tubule and plays a key role in regulating blood pressure. Loss of function of NCC results in Gitelman's syndrome, a renal salt wasting disorder, with many patients suffering from arterial hypotension [99]. The chloride channel CLC-K1 mediates transepithelial transport of chloride in the thin ascending limb in the inner medulla [100]. CLC-K1 null mice displayed polyuria and an increase in urine volume along with a decreased urine osmolality compared to their wild-type counterparts [101]. The renal outer medullary potassium ($K^+$) channel (ROMK) is expressed in the thick ascending limb, macula densa, distal convoluted tubule, connecting tubule and CD and secretes $K^+$ into the tubular lumen. ROMK-KO mice displayed severe dehydration by one week of age, displayed growth retardation, and only 5% survived until adulthood [102]. Adult ROMK null mice manifested lower blood pressure and urine osmolality, along with higher $Na^+$ and $K^+$ excretion and increased water consumption compared to the wild-type mice [103].

### *6.5.9. Non-coding RNAs*

Emerging studies in vertebrates indicate a pivotal role for non-coding RNA, particularly micro RNAs (miRNAs), in the regulation of osmotic and water fluid homeostasis. MicroRNAs are transcribed from the genomic DNA, resulting in a transcript called pri-miRNA, which is trimmed into pre-miRNA by a nuclear complex involving the enzyme RNase III endonucleases Drosha and DiGeorge syndrome Critical Region 8 (DGCR8). Pre-miRNAs are transported into the cytoplasm *via* Exportin-5, and are further processed by Dicer RNase into a miRNA duplex. The duplex unwinds, and the mature miRNA (~ 22 nucleotides long, the guide RNA) assembles into RNA-induced silencing complex (RISC), which contains argonaute 2 (ARG2) and transactivating response RNA-binding protein 1 (TRBP1). The other miRNA strand (passenger strand) is degraded. The mature miRNA base pairs with target mRNA to direct gene silencing through mRNA cleavage or translation repression (Fig. **6.5**) [104 - 106]. Recent studies in plants and mammals have shown that water deprivation affects miRNA biogenesis machinery and that miRNAs regulate the expression of ion and water channels as well as neurohumoral factors, including ADH involved in water and electrolyte balance. For instance, Tian *et al*. [107] identified 17 miRNAs that were differentially expressed in the renal cortex and medulla of rodents. In response to

high NaCl exposure in cultured renal medullary epithelial cells, Huang *et al*. [108] reported rapid and significant changes of miRNA profiles and expressions, indicating potential roles of renal miRNAs in osmotic stress responses. Specifically, it has been shown that miRNAs targets ion channels and transporters. For example, miR-192 was found to regulate the $Na^+/K^+$ ATPase (ATP1β1), which is the driving force of tubular transport in the kidney [109]. Tobon and colleagues [110], reported that miR-142-3p regulate renal D1 dopamine receptor which modulates the $Na^+/K^+$ ATPase and the $Na^+/H^+$ exchangers and thereby regulates diuresis and natriuresis. Marques *et al*. [111] have shown that miR-181 modulates RAAS system *via* regulation of renin expression in the human hypertensive kidney. Similarly, conditional deletion of Dicer1 decreased renin expression and severely reduced the number of juxtaglomerular cells along with low blood pressure [112]. miR-421 was found to be a down regulator of ACE2, which hydrolyses ANGII to ANG(1-7) [113]. Further functional studies conducted by Jin *et al*. [114] showed that miR-132 attenuates ANGII-induced CREB activation, and this effect was mediated *via* AT1R. Other studies have shown in rodents that salt depletion, potassium load, or chronic aldosterone infusion down regulate miR-192 expression [115]. Transgenic mice with overexpression of miR-466a-3p displayed polydipsia, polyuria, ion homeostasis dysregulation, and altered kidney morphology [116]. In another study by the same group, exposure to sodium chloride down regulated miR-200b and miR-717 expression, and these miRNAs regulate the expression of the transcription factor osmotic response element-binding protein (OREBP), demonstrating their critical roles in cellular osmoresponses.

In a study using birds non-mammalian species, our group have shown that water restriction for 3h up-regulated the renal expression of AQP2, AQP3, AQP4, ADH, ATP1β1, and miRNA biogenesis machinery (Dicer1, Arg2, DGCR8, and TRBP1) in a population-dependent manner [117].

## 6.6. Interaction Between Hunger- and Thirst-motivational Drives

Based on the principle of "singleness of action", animals need to choose a specific behavior over another and this depends on the physiological conditions, resources availability, and environmental conditions. One might ask the question how hunger and thirst motivational drives interact? And if the animal needs to choose, then which one overrides the other? In flies, genetically defined four interoceptive neurons in the subesophageal zone are activated under hunger states and inhibited under thirst states [118]. Furthermore, stimulation of these neuron populations induced sugar consumption and inhibited water intake. In rodents, activation of AgRP neurons has been reported to suppress competing drives such as thirst, pain,

fear, and territory marking [119 - 121]. The integration mechanism of hunger and thirst has been tested in an elegant study conducted by Takei *et al*. [122] using the Spinifex hopping (*Notomys alexis*) mice, in which water deprivation for either 12 or 29 days induced a biphasic pattern of feed intake. An initial hypophagia was followed by a sustained increase in appetite for the latter phase of water restriction that was above that of water-replete mice. In the early phase of water deprivation, the changes in feed intake were driven by appropriate changes in plasma leptin and ghrelin however the sustained feed intake during the later phase of water restriction occurred despite an increase in circulating leptin that should act as an anorexigenic hormone. This study suggests that the signals controlling thirst and water intake might override the signals controlling appetite, hunger, and feed intake, which might be supported by the need for substrates for metabolic water. Furthermore, a switch of metabolic strategy from lipids mobilization to carbohydrate storage was observed, indicating potential enhancement of metabolic water production per oxygen molecule [122].

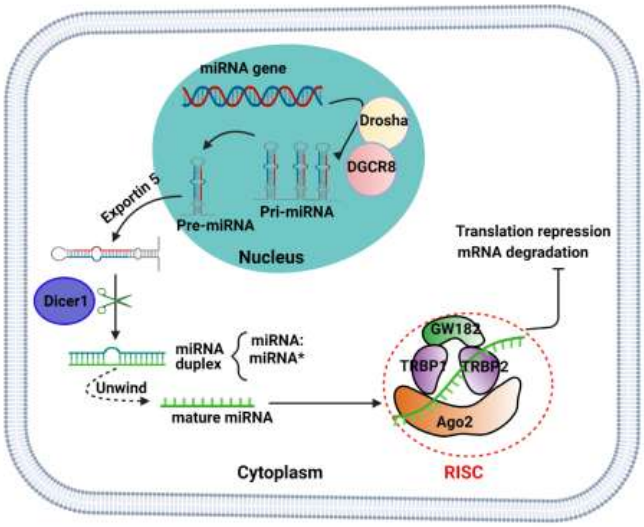


**Fig. (6.5). Schematic illustration of miRNA biogenesis.** miRNA biogenesis begins in the nucleus, where RNA-polymerase II-dependent (RNAPII) transcription of a relatively large capped and polyadenylated transcript known as primary miRNA (pri-miRNA). Pri-miRNA is processed by the RNase III endonuclease, Drosha, and its cofactor, DGCR8, into smaller stem-looped structures known as precursor miRNAs (pre-miRNA). Pre-miRNAs are transported out of the nucleus by Exportin 5 into the cytoplasm, where further processing by a second RNase III enzyme, Dicer 1, leads to the generation of mature miRNA. The mature miRNA is associated with the miRNA-induced silencing complex (miRISC), where Watson-Crick base-pairing between the seed-sequence of a mature miRNA and complementary sequences primarily located within 3'-UTRs of mRNAs results in post-transcriptional gene silencing. Ago 2, argonaute 2; DGCR8, DiGeorge syndrome Critical Region 8; TRBP1/2, transactivating response RNA-binding protein.

## CONCLUSION

Animals must drink sufficient water and ingest food from external sources to

maintain water homeostasis and compensate for water losses. Water intake is regulated at both central and peripheral levels and involves neurochemical circuits and peripheral osmoreceptors. At molecular levels, several signaling pathways, such as RAAS, ADH, AQPs, TRPV, ncRNA, and several others involved in water homeostasis regulation have been discovered and discussed in this chapter.

## NOTES

[1] Osmosis: is a spontaneous movement of solvent molecules through a selectively permeable membrane, from a less concentrated to a higher concentration, to equalize the solute concentration on each side of the membrane.

[2] Hyponatremia occurs when the concentration of sodium in blood is abnormally low

[3] Hypovolemia is an abnormal decrease in the volume of blood plasma which can occur with dehydration or bleeding

[4] Hypernatremia is a rise in serum sodium concentration caused by a decrease in total body water

[5] Polyuria is a production of abnormally large volume of dilute urine.

[6] Polydipsia is a constant excessive drinking due to thirst

[7] CSF, cerebrospinal fluid

[8] ADH, antidiuretic hormone

## REFERENCES

[1] Lewis SA. A reinvestigation of the function of the mammalian urinary bladder. Am J Physiol 1977; 232(3): F187-95.
[PMID: 320880]

[2] Cameron P, Hiroi M, Ngai J, Scott K. The molecular basis for water taste in Drosophila. Nature 2010; 465(7294): 91-5.
[http://dx.doi.org/10.1038/nature09011] [PMID: 20364123]

[3] Chen Z, Wang Q, Wang Z. The amiloride-sensitive epithelial Na+ channel PPK28 is essential for drosophila gustatory water reception. J Neurosci 2010; 30(18): 6247-52.
[http://dx.doi.org/10.1523/JNEUROSCI.0627-10.2010] [PMID: 20445050]

[4] Lin S, Owald D, Chandra V, Talbot C, Huetteroth W, Waddell S. Neural correlates of water reward in thirsty Drosophila. Nat Neurosci 2014; 17(11): 1536-42.
[http://dx.doi.org/10.1038/nn.3827] [PMID: 25262493]

[5] Zotterman Y. Species differences in the water taste. Acta Physiol Scand 1956; 37(1): 60-70.
[http://dx.doi.org/10.1111/j.1748-1716.1956.tb01342.x] [PMID: 13339453]

[6] Zocchi D, Wennemuth G, Oka Y. The cellular mechanism for water detection in the mammalian taste system. Nat Neurosci 2017; 20(7): 927-33.
[http://dx.doi.org/10.1038/nn.4575] [PMID: 28553944]

[7] Oka Y, Butnaru M, von Buchholtz L, Ryba NJP, Zuker CS. High salt recruits aversive taste pathways. Nature 2013; 494(7438): 472-5.
[http://dx.doi.org/10.1038/nature11905] [PMID: 23407495]

[8] Chandrashekar J, Kuhn C, Oka Y, *et al.* The cells and peripheral representation of sodium taste in mice. Nature 2010; 464(7286): 297-301.
[http://dx.doi.org/10.1038/nature08783] [PMID: 20107438]

[9] Shigemura N, Ohkuri T, Sadamitsu C, *et al.* Amiloride-sensitive NaCl taste responses are associated with genetic variation of ENaC α-subunit in mice. Am J Physiol Regul Integr Comp Physiol 2008; 294(1): R66-75.
[http://dx.doi.org/10.1152/ajpregu.00420.2007] [PMID: 17977920]

[10] McKenna K, Thompson C. Osmoregulation in clinical disorders of thirst appreciation. Clin Endocrinol (Oxf) 1998; 49(2): 139-52.
[http://dx.doi.org/10.1046/j.1365-2265.1998.00572.x] [PMID: 9828896]

[11] Dunn FL, Brennan TJ, Nelson AE, Robertson GL. The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J Clin Invest 1973; 52(12): 3212-9.
[http://dx.doi.org/10.1172/JCI107521] [PMID: 4750450]

[12] Geelen G, Keil LC, Kravik SE, *et al.* Inhibition of plasma vasopressin after drinking in dehydrated humans. Am J Physiol 1984; 247(6 Pt 2): R968-71.
[PMID: 6507654]

[13] Andersen LJ, Jensen TUS, Bestle MH, Bie P. Gastrointestinal osmoreceptors and renal sodium excretion in humans. Am J Physiol Regul Integr Comp Physiol 2000; 278(2): R287-94.
[http://dx.doi.org/10.1152/ajpregu.2000.278.2.R287] [PMID: 10666127]

[14] Bourque CW. Central mechanisms of osmosensation and systemic osmoregulation. Nat Rev Neurosci 2008; 9(7): 519-31.
[http://dx.doi.org/10.1038/nrn2400] [PMID: 18509340]

[15] Verney EB. Croonian Lecture - The antidiuretic hormone and the factors which determine its release. Proc R Soc Lond B Biol Sci 1947; 135(878): 25-106.
[http://dx.doi.org/10.1098/rspb.1947.0037] [PMID: 18918876]

[16] Buggy J, Hoffman WE, Phillips MI, Fisher AE, Johnson AK. Osmosensitivity of rat third ventricle and interactions with angiotensin. Am J Physiol 1979; 236(1): R75-82.
[PMID: 434190]

[17] McKinley MJ, Mathai ML, McAllen RM, *et al.* Vasopressin secretion: osmotic and hormonal regulation by the lamina terminalis. J Neuroendocrinol 2004; 16(4): 340-7.
[http://dx.doi.org/10.1111/j.0953-8194.2004.01184.x] [PMID: 15089972]

[18] McKinley MJ, Mathai ML, Pennington G, Rundgren M, Vivas L. Effect of individual or combined ablation of the nuclear groups of the lamina terminalis on water drinking in sheep. Am J Physiol 1999; 276(3): R673-83.
[PMID: 10070127]

[19] Bourque CW, Ciura S, Trudel E, Stachniak TJE, Sharif-Naeini R. Neurophysiological characterization

of mammalian osmosensitive neurones. Exp Physiol 2007; 92(3): 499-505.
[http://dx.doi.org/10.1113/expphysiol.2006.035634] [PMID: 17350993]

[20] Shi P, Martinez MA, Calderon AS, Chen Q, Cunningham JT, Toney GM. Intra-carotid hyperosmotic stimulation increases Fos staining in forebrain organum vasculosum laminae terminalis neurones that project to the hypothalamic paraventricular nucleus. J Physiol 2008; 586(21): 5231-45.
[http://dx.doi.org/10.1113/jphysiol.2008.159665] [PMID: 18755745]

[21] Ferguson AV, Bains JS. Electrophysiology of the circumventricular organs. Front Neuroendocrinol 1996; 17(4): 440-75.
[http://dx.doi.org/10.1006/frne.1996.0012] [PMID: 8905349]

[22] Simpson JB, Epstein AN, Camardo JS Jr. Localization of receptors for the dipsogenic action of angiotensin II in the subfornical organ of rats. J Comp Physiol Psychol 1978; 92(4): 581-608.
[http://dx.doi.org/10.1037/h0077503] [PMID: 211148]

[23] Oka Y, Ye M, Zuker CS. Thirst driving and suppressing signals encoded by distinct neural populations in the brain. Nature 2015; 520(7547): 349-52.
[http://dx.doi.org/10.1038/nature14108] [PMID: 25624099]

[24] Kuramochi G, Kobayashi I. Regulation of the urine concentration mechanism by the oropharyngeal afferent pathway in man. Am J Nephrol 2000; 20(1): 42-7.
[http://dx.doi.org/10.1159/000013554] [PMID: 10644867]

[25] Carlson SH, Beitz A, Osborn JW. Intragastric hypertonic saline increases vasopressin and central Fos immunoreactivity in conscious rats. Am J Physiol 1997; 272(3 Pt 2): R750-8.
[PMID: 9087636]

[26] Baertschi AJ, Vallet PG. Osmosensitivity of the hepatic portal vein area and vasopressin release in rats. J Physiol 1981; 315(1): 217-30.
[http://dx.doi.org/10.1113/jphysiol.1981.sp013743] [PMID: 7310708]

[27] Adachi A, Niijima A, Jacobs HL. An hepatic osmoreceptor mechanism in the rat: electrophysiological and behavioral studies. Am J Physiol 1976; 231(4): 1043-9.
[http://dx.doi.org/10.1152/ajplegacy.1976.231.4.1043] [PMID: 984191]

[28] Choi-Kwon S, Baertschi AJ. Splanchnic osmosensation and vasopressin: mechanisms and neural pathways. Am J Physiol 1991; 261(1 Pt 1): E18-25.
[PMID: 1858871]

[29] Baertschi AJ, Pence RA. Gut-brain signaling of water absorption inhibits vasopressin in rats. Am J Physiol 1995; 268(1 Pt 2): R236-47.
[PMID: 7840327]

[30] Niijima A. Afferent discharges from osmoreceptors in the liver of the guinea pig. Science 1969; 166(3912): 1519-20.
[http://dx.doi.org/10.1126/science.166.3912.1519] [PMID: 17655046]

[31] Ciura S, Bourque CW. Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. J Neurosci 2006; 26(35): 9069-75.
[http://dx.doi.org/10.1523/JNEUROSCI.0877-06.2006] [PMID: 16943565]

[32] Clapham DE. SnapShot: Mammalian TRP Channels. Cell 2007; 129(1): 220.e1-2.
[http://dx.doi.org/10.1016/j.cell.2007.03.034] [PMID: 17418797]

[33] Liedtke W, Choe Y, Martí-Renom MA, *et al.* Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 2000; 103(3): 525-35.
[http://dx.doi.org/10.1016/S0092-8674(00)00143-4] [PMID: 11081638]

[34] Liedtke W, Friedman JM. Abnormal osmotic regulation in trpv4-/- mice. Proc Natl Acad Sci USA 2003; 100(23): 13698-703.
[http://dx.doi.org/10.1073/pnas.1735416100] [PMID: 14581612]

[35] Tsushima H, Mori M. Antidipsogenic effects of a TRPV4 agonist, 4α-phorbol 12,13-didecanoate, injected into the cerebroventricle. Am J Physiol Regul Integr Comp Physiol 2006; 290(6): R1736-41. [http://dx.doi.org/10.1152/ajpregu.00043.2005] [PMID: 16439673]

[36] Mizuno A, Matsumoto N, Imai M, Suzuki M. Impaired osmotic sensation in mice lacking TRPV4. Am J Physiol Cell Physiol 2003; 285(1): C96-C101. [http://dx.doi.org/10.1152/ajpcell.00559.2002] [PMID: 12777254]

[37] Naeini RS, Witty MF, Séguéla P, Bourque CW. An N-terminal variant of Trpv1 channel is required for osmosensory transduction. Nat Neurosci 2006; 9(1): 93-8. [http://dx.doi.org/10.1038/nn1614] [PMID: 16327782]

[38] Taylor AC, McCarthy JJ, Stocker SD. Mice lacking the transient receptor vanilloid potential 1 channel display normal thirst responses and central Fos activation to hypernatremia. Am J Physiol Regul Integr Comp Physiol 2008; 294(4): R1285-93. [http://dx.doi.org/10.1152/ajpregu.00003.2008] [PMID: 18272658]

[39] Nedungadi TP, Carreño FR, Walch JD, Bathina CS, Cunningham JT. Region-specific changes in transient receptor potential vanilloid channel expression in the vasopressin magnocellular system in hepatic cirrhosis-induced hyponatraemia. J Neuroendocrinol 2012; 24(4): 642-52. [http://dx.doi.org/10.1111/j.1365-2826.2011.02273.x] [PMID: 22188460]

[40] Wainwright A, Rutter AR, Seabrook GR, Reilly K, Oliver KR. Discrete expression of TRPV2 within the hypothalamo-neurohypophysial system: Implications for regulatory activity within the hypothalamic-pituitary-adrenal axis. J Comp Neurol 2004; 474(1): 24-42. [http://dx.doi.org/10.1002/cne.20100] [PMID: 15156577]

[41] Nedungadi TP, Dutta M, Bathina CS, Caterina MJ, Cunningham JT. Expression and distribution of TRPV2 in rat brain. Exp Neurol 2012; 237(1): 223-37. [http://dx.doi.org/10.1016/j.expneurol.2012.06.017] [PMID: 22750329]

[42] Nehme A, Zouein FA, Zayeri ZD, Zibara K. An Update on the Tissue Renin Angiotensin System and Its Role in Physiology and Pathology. J Cardiovasc Dev Dis 2019; 6(2): 14. [http://dx.doi.org/10.3390/jcdd6020014] [PMID: 30934934]

[43] Epstein AN, Fitzsimons JT, Rolls BJ. Drinking induced by injection of angiotensin into the brain of the rat. J Physiol 1970; 210(2): 457-74. [http://dx.doi.org/10.1113/jphysiol.1970.sp009220] [PMID: 4322723]

[44] Harrison-Bernard LM. The renal renin-angiotensin system. Adv Physiol Educ 2009; 33(4): 270-4. [http://dx.doi.org/10.1152/advan.00049.2009] [PMID: 19948673]

[45] Ganten D, Marquez-Julio A, Granger P, *et al.* Renin in dog brain. Am J Physiol 1971; 221(6): 1733-7. [http://dx.doi.org/10.1152/ajplegacy.1971.221.6.1733] [PMID: 4330904]

[46] Boone M, Deen PMT. Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. Pflugers Arch 2008; 456(6): 1005-24. [http://dx.doi.org/10.1007/s00424-008-0498-1] [PMID: 18431594]

[47] Robertson GL. The regulation of vasopressin function in health and disease. Recent Prog Horm Res 1976; 33: 333-85. [PMID: 801194]

[48] de Arruda Camargo LA, Saad WA, Cerri PS. Effects of V1 and angiotensin receptor subtypes of the paraventricular nucleus on the water intake induced by vasopressin injected into the lateral septal area. Brain Res Bull 2003; 61(5): 481-7. [http://dx.doi.org/10.1016/S0361-9230(03)00184-9] [PMID: 13679246]

[49] Thibonnier M, Coles P, Thibonnier A, Shoham M. Molecular pharmacology and modeling of vasopressin receptors. Prog Brain Res 2002; 139: 179-96. [http://dx.doi.org/10.1016/S0079-6123(02)39016-2] [PMID: 12436935]

[50] Fushimi K, Uchida S, Harat Y, Hirata Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. Nature 1993; 361(6412): 549-52. [http://dx.doi.org/10.1038/361549a0] [PMID: 8429910]

[51] Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. Proc Natl Acad Sci USA 1995; 92(4): 1013-7. [http://dx.doi.org/10.1073/pnas.92.4.1013] [PMID: 7532304]

[52] Fenton RA. Essential role of vasopressin-regulated urea transport processes in the mammalian kidney. Pflugers Arch 2009; 458(1): 169-77. [http://dx.doi.org/10.1007/s00424-008-0612-4] [PMID: 19011892]

[53] Brooks FP, Pickford M. The effect of posterior pituitary hormones on the excretion of electrolytes, in dogs. J Physiol 1958; 142(3): 468-93. [http://dx.doi.org/10.1113/jphysiol.1958.sp006031] [PMID: 13576449]

[54] Li C, Wang W, Summer SN, *et al.* Molecular mechanisms of antidiuretic effect of oxytocin. J Am Soc Nephrol 2008; 19(2): 225-32. [http://dx.doi.org/10.1681/ASN.2007010029] [PMID: 18057218]

[55] Bernal A, Mahía J, Puerto A. Oxytocin, water intake, and food sodium availability in male rats. Horm Behav 2007; 52(3): 289-96. [http://dx.doi.org/10.1016/j.yhbeh.2007.05.005] [PMID: 17559853]

[56] Terashima Y, Kondo K, Oiso Y. Administration of oxytocin affects vasopressin V2 receptor and aquaporin-2 gene expression in the rat. Life Sci 1999; 64(16): 1447-53. [http://dx.doi.org/10.1016/S0024-3205(99)00078-8] [PMID: 10321724]

[57] Jeon US, Joo KW, Na KY, *et al.* Oxytocin induces apical and basolateral redistribution of aquaporin-2 in rat kidney. Nephron, Exp Nephrol 2003; 93(1): e36-45. [http://dx.doi.org/10.1159/000066651] [PMID: 12411748]

[58] Villanger O, Veel T, Ræder MG. Secretin causes H+/HCO3− secretion from pig pancreatic ductules by vacuolar-type H+-adenosine triphosphatase. Gastroenterology 1995; 108(3): 850-9. [http://dx.doi.org/10.1016/0016-5085(95)90460-3] [PMID: 7875488]

[59] Marinelli RA, Pham L, Agre P, LaRusso NF. Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. Evidence for a secretin-induced vesicular translocation of aquaporin-1. J Biol Chem 1997; 272(20): 12984-8. [http://dx.doi.org/10.1074/jbc.272.20.12984] [PMID: 9148905]

[60] Chow BKC, Cheung KH, Tsang EMW, Leung MCT, Lee SMY, Wong PYD. Secretin controls anion secretion in the rat epididymis in an autocrine/paracrine fashion. Biol Reprod 2004; 70(6): 1594-9. [http://dx.doi.org/10.1095/biolreprod.103.024257] [PMID: 14749298]

[61] Viteri AL, Poppell JW, Lasater JM, Dyck WP. Renal response to secretin. J Appl Physiol 1975; 38(4): 661-4. [http://dx.doi.org/10.1152/jappl.1975.38.4.661] [PMID: 237865]

[62] Waldum HL, Sundsfjord JA, Aanstad U, Burhol PG. The effect of secretin on renal haemodynamics in man. Scand J Clin Lab Invest 1980; 40(5): 475-8. [http://dx.doi.org/10.3109/00365518009101870] [PMID: 7444349]

[63] Chu JYS, Chung SCK, Lam AKM, Tam S, Chung SK, Chow BKC. Phenotypes developed in secretin receptor-null mice indicated a role for secretin in regulating renal water reabsorption. Mol Cell Biol 2007; 27(7): 2499-511. [http://dx.doi.org/10.1128/MCB.01088-06] [PMID: 17283064]

[64] Oektedalen O, Opstad PK, de Muckadell OBS. Secretin—a new stress hormone? Regul Pept 1982; 4(4): 213-9. [http://dx.doi.org/10.1016/0167-0115(82)90113-6] [PMID: 7146493]

[65] Motojima Y, Kawasaki M, Matsuura T, *et al.* Effects of peripherally administered cholecystokinin-8 and secretin on feeding/drinking and oxytocin-mRFP1 fluorescence in transgenic rats. Neurosci Res 2016; 109: 63-9.
[http://dx.doi.org/10.1016/j.neures.2016.02.005] [PMID: 26919961]

[66] Lee VHY, Lee LTO, Chu JYS, *et al.* An indispensable role of secretin in mediating the osmoregulatory functions of angiotensin II. FASEB J 2010; 24(12): 5024-32.
[http://dx.doi.org/10.1096/fj.10-165399] [PMID: 20739612]

[67] Cheng CYY, Chu JYS, Chow BKC. Vasopressin-independent mechanisms in controlling water homeostasis. J Mol Endocrinol 2009; 43(3): 81-92.
[http://dx.doi.org/10.1677/JME-08-0123] [PMID: 19318428]

[68] Twarog BM, Page IH. Serotonin content of some mammalian tissues and urine and a method for its determination. Am J Physiol 1953; 175(1): 157-61.
[http://dx.doi.org/10.1152/ajplegacy.1953.175.1.157] [PMID: 13114371]

[69] Molliver M. Serotonergic neuronal systems: what their anatomic organization tells us about function. J Clin Psychopharmacol 1987; 7(6) (Suppl. 6): 24S.
[http://dx.doi.org/10.1097/00004714-198712001-00002] [PMID: 3323265]

[70] Tangaprégassom AM, Tangaprégassom MJ, Soulairac A. [Proceedings: Effects of the raphe system lesioning on water intake and anterior hypothalamic neurosecretion of the rat (author's transl)]. Ann Endocrinol (Paris) 1974; 35(6): 667-8. [Proceedings: Effects of the raphe system lesioning on water intake and anterior hypothalamic neurosecretion of the rat (author's transl)].
[PMID: 4463815]

[71] Cavalcante-Lima HR, Lima HRC, Costa-e-Sousa RH, *et al.* Dipsogenic stimulation in ibotenic DRN-lesioned rats induces concomitant sodium appetite. Neurosci Lett 2005; 374(1): 5-10.
[http://dx.doi.org/10.1016/j.neulet.2004.10.017] [PMID: 15631886]

[72] Simansky KJ. Peripheral 5-carboxamidotryptamine (5-CT) elicits drinking by stimulating 5-HT1-like serotonergic receptors in rats. Pharmacol Biochem Behav 1991; 38(2): 459-62.
[http://dx.doi.org/10.1016/0091-3057(91)90306-M] [PMID: 1829233]

[73] Rowland NE, Li BH, Fregly MJ, Smith GC. Involvement of angiotensin in water intake induced by peripheral administration of a serotonin agonist, 5-carboxyamidotryptamine. Brain Res 1994; 664(1-2): 148-54.
[http://dx.doi.org/10.1016/0006-8993(94)91965-8] [PMID: 7895024]

[74] DE CASTRO-e-SILVA E, Sarmento C, Nascimento TA, *et al.* Effect of third ventricle administration of L-694,247, a selective 5-HT1D receptor agonist, on water intake in rats. Pharmacol Biochem Behav 1997; 57(4): 749-54.
[http://dx.doi.org/10.1016/S0091-3057(96)00457-1] [PMID: 9259002]

[75] de Arruda Camargo GMP, de Arruda Camargo LA, Saad WA. On a possible dual role for the lateral septal area 5-HT1A receptor system in the regulation of water intake and urinary excretion. Behav Brain Res 2010; 215(1): 122-8.
[http://dx.doi.org/10.1016/j.bbr.2010.07.010] [PMID: 20638418]

[76] Villa PS, Menani JV, Camargo GMPA, Camargo LAA, Saad WA. Activation of the serotonergic 5-HT1A receptor in the paraventricular nucleus of the hypothalamus inhibits water intake and increases urinary excretion in water-deprived rats. Regul Pept 2008; 150(1-3): 14-20.
[http://dx.doi.org/10.1016/j.regpep.2008.05.003] [PMID: 18556076]

[77] Reis LC, Ramalho MJ, Antunes-Rodrigues J. Central serotonergic modulation of drinking behavior induced by water deprivation: effect of a serotonergic agonist (MK-212) administered intracerebroventricularly. Braz J Med Biol Res 1990; 23(12): 1335-8.
[PMID: 2136566]

[78] Castro L, Maldonado I, Campos I, *et al.* Central administration of mCPP, a serotonin 5-HT2B/2C

agonist, decreases water intake in rats. Pharmacol Biochem Behav 2002; 72(4): 891-8.
[http://dx.doi.org/10.1016/S0091-3057(02)00763-3] [PMID: 12062579]

[79] Castro L, Varjão B, Silva I, *et al.* Effect of the intracerebroventricular administration of GR 113808, a selective 5-HT4 antagonist, on water intake during hyperosmolarity and hypovolemia. Braz J Med Biol Res 2001; 34(6): 791-6.
[http://dx.doi.org/10.1590/S0100-879X2001000600015] [PMID: 11378670]

[80] Castro L, Athanazio R, Barbetta M, *et al.* Central 5-HT2B/2C and 5-HT3 receptor stimulation decreases salt intake in sodium-depleted rats. Brain Res 2003; 981(1-2): 151-9.
[http://dx.doi.org/10.1016/S0006-8993(03)03015-4] [PMID: 12885436]

[81] Neill JC, Cooper SJ. Selective reduction by serotonergic agents of hypertonic saline consumption in rats: evidence for possible 5-HT1C receptor mediation. Psychopharmacology (Berl) 1989; 99(2): 196-201.
[http://dx.doi.org/10.1007/BF00442807] [PMID: 2508154]

[82] Rouah-Rosilio M, Orosco M, Nicolaidis S. Serotoninergic modulation of sodium appetite in the rat. Physiol Behav 1994; 55(5): 811-6.
[http://dx.doi.org/10.1016/0031-9384(94)90064-7] [PMID: 8022898]

[83] Kuhn DM, Wolf WA, Lovenberg W. Review of the role of the central serotonergic neuronal system in blood pressure regulation. Hypertension 1980; 2(3): 243-55.
[http://dx.doi.org/10.1161/01.HYP.2.3.243] [PMID: 6446522]

[84] Verkman AS. Aquaporins. Curr Biol 2013; 23(2): R52-5.
[http://dx.doi.org/10.1016/j.cub.2012.11.025] [PMID: 23347934]

[85] Gonen T, Sliz P, Kistler J, Cheng Y, Walz T. Aquaporin-0 membrane junctions reveal the structure of a closed water pore. Nature 2004; 429(6988): 193-7.
[http://dx.doi.org/10.1038/nature02503] [PMID: 15141214]

[86] Denker BM, Smith BL, Kuhajda FP, Agre P. Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. J Biol Chem 1988; 263(30): 15634-42.
[http://dx.doi.org/10.1016/S0021-9258(19)37635-5] [PMID: 3049610]

[87] Preston GM, Agre P. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. Proc Natl Acad Sci USA 1991; 88(24): 11110-4.
[http://dx.doi.org/10.1073/pnas.88.24.11110] [PMID: 1722319]

[88] Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. J Biol Chem 1998; 273(8): 4296-9.
[http://dx.doi.org/10.1074/jbc.273.8.4296] [PMID: 9468475]

[89] Ma T, Song Y, Yang B, *et al.* Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. Proc Natl Acad Sci USA 2000; 97(8): 4386-91.
[http://dx.doi.org/10.1073/pnas.080499597] [PMID: 10737773]

[90] Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. J Clin Invest 1997; 100(5): 957-62.
[http://dx.doi.org/10.1172/JCI231] [PMID: 9276712]

[91] Meli R, Pirozzi C, Pelagalli A. New Perspectives on the Potential Role of Aquaporins (AQPs) in the Physiology of Inflammation. Front Physiol 2018; 9: 101.
[http://dx.doi.org/10.3389/fphys.2018.00101] [PMID: 29503618]

[92] Lorenz JN, Schultheis PJ, Traynor T, Shull GE, Schnermann J. Micropuncture analysis of single-nephron function in NHE3-deficient mice. Am J Physiol 1999; 277(3): F447-53.
[PMID: 10484528]

[93] Amlal H, Ledoussal C, Sheriff S, Shull GE, Soleimani M. Downregulation of renal AQP2 water channel and NKCC2 in mice lacking the apical $Na^+$ □$H^+$ exchanger NHE3. J Physiol 2003; 553(2): 511-22.
[http://dx.doi.org/10.1113/jphysiol.2003.053363] [PMID: 14500765]

[94] Oppermann M, Mizel D, Huang G, *et al.* Macula densa control of renin secretion and preglomerular resistance in mice with selective deletion of the B isoform of the Na,K,2Cl co-transporter. J Am Soc Nephrol 2006; 17(8): 2143-52.
[http://dx.doi.org/10.1681/ASN.2006040384] [PMID: 16807402]

[95] Takahashi N, Chernavvsky DR, Gomez RA, Igarashi P, Gitelman HJ, Smithies O. Uncompensated polyuria in a mouse model of Bartter's syndrome. Proc Natl Acad Sci USA 2000; 97(10): 5434-9.
[http://dx.doi.org/10.1073/pnas.090091297] [PMID: 10779555]

[96] Hanukoglu I, Hanukoglu A. Epithelial sodium channel (ENaC) family: Phylogeny, structure–function, tissue distribution, and associated inherited diseases. Gene 2016; 579(2): 95-132.
[http://dx.doi.org/10.1016/j.gene.2015.12.061] [PMID: 26772908]

[97] Barker PM, Nguyen MS, Gatzy JT, *et al.* Role of gammaENaC subunit in lung liquid clearance and electrolyte balance in newborn mice. Insights into perinatal adaptation and pseudohypoaldosteronism. J Clin Invest 1998; 102(8): 1634-40.
[http://dx.doi.org/10.1172/JCI3971] [PMID: 9788978]

[98] Rubera I, Loffing J, Palmer LG, *et al.* Collecting duct–specific gene inactivation of αENaC in the mouse kidney does not impair sodium and potassium balance. J Clin Invest 2003; 112(4): 554-65.
[http://dx.doi.org/10.1172/JCI16956] [PMID: 12925696]

[99] Simon DB, Nelson-Williams C, Johnson Bia M, *et al.* Gitelman's variant of Barter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na–Cl cotransporter. Nat Genet 1996; 12(1): 24-30.
[http://dx.doi.org/10.1038/ng0196-24] [PMID: 8528245]

[100] Uchida S, Sasaki S, Nitta K, *et al.* Localization and functional characterization of rat kidney-specific chloride channel, ClC-K1. J Clin Invest 1995; 95(1): 104-13.
[http://dx.doi.org/10.1172/JCI117626] [PMID: 7814604]

[101] Matsumura Y, Uchida S, Kondo Y, *et al.* Overt nephrogenic diabetes insipidus in mice lacking the CLC-K1 chloride channel. Nat Genet 1999; 21(1): 95-8.
[http://dx.doi.org/10.1038/5036] [PMID: 9916798]

[102] Lorenz JN, Baird NR, Judd LM, *et al.* Impaired renal NaCl absorption in mice lacking the ROMK potassium channel, a model for type II Bartter's syndrome. J Biol Chem 2002; 277(40): 37871-80.
[http://dx.doi.org/10.1074/jbc.M205627200] [PMID: 12122007]

[103] Lu M, Wang T, Yan Q, *et al.* Absence of small conductance K+ channel (SK) activity in apical membranes of thick ascending limb and cortical collecting duct in ROMK (Bartter's) knockout mice. J Biol Chem 2002; 277(40): 37881-7.
[http://dx.doi.org/10.1074/jbc.M206644200] [PMID: 12130653]

[104] Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. Cell 2008; 132(1): 9-14.
[http://dx.doi.org/10.1016/j.cell.2007.12.024] [PMID: 18191211]

[105] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9(2): 102-14.
[http://dx.doi.org/10.1038/nrg2290] [PMID: 18197166]

[106] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136(2): 215-33.
[http://dx.doi.org/10.1016/j.cell.2009.01.002] [PMID: 19167326]

[107] Tian Z, Greene AS, Pietrusz JL, Matus IR, Liang M. MicroRNA–target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. Genome Res 2008; 18(3):

404-11.
[http://dx.doi.org/10.1101/gr.6587008] [PMID: 18230805]

[108] Huang W, Liu H, Wang T, *et al.* Tonicity-responsive microRNAs contribute to the maximal induction of osmoregulatory transcription factor OREBP in response to high-NaCl hypertonicity. Nucleic Acids Res 2011; 39(2): 475-85.
[http://dx.doi.org/10.1093/nar/gkq818] [PMID: 20852262]

[109] Mladinov D, Liu Y, Mattson DL, Liang M. MicroRNAs contribute to the maintenance of cell-typ--specific physiological characteristics: miR-192 targets Na+/K+-ATPase β1. Nucleic Acids Res 2013; 41(2): 1273-83.
[http://dx.doi.org/10.1093/nar/gks1228] [PMID: 23221637]

[110] Tobón KE, Chang D, Kuzhikandathil EV. MicroRNA 142-3p mediates post-transcriptional regulation of D1 dopamine receptor expression. PLoS One 2012; 7(11)e49288
[http://dx.doi.org/10.1371/journal.pone.0049288] [PMID: 23152889]

[111] Marques FZ, Campain AE, Tomaszewski M, *et al.* Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs. Hypertension 2011; 58(6): 1093-8.
[http://dx.doi.org/10.1161/HYPERTENSIONAHA.111.180729] [PMID: 22042811]

[112] Sequeira-Lopez MLS, Weatherford ET, Borges GR, *et al.* The microRNA-processing enzyme dicer maintains juxtaglomerular cells. J Am Soc Nephrol 2010; 21(3): 460-7.
[http://dx.doi.org/10.1681/ASN.2009090964] [PMID: 20056748]

[113] Lambert DW, Lambert LA, Clarke NE, Hooper NM, Porter KE, Turner AJ. Angiotensin-converting enzyme 2 is subject to post-transcriptional regulation by *miR-421*. Clin Sci (Lond) 2014; 127(4): 243-9.
[http://dx.doi.org/10.1042/CS20130420] [PMID: 24564768]

[114] Jin W, Reddy MA, Chen Z, *et al.* Small RNA sequencing reveals microRNAs that modulate angiotensin II effects in vascular smooth muscle cells. J Biol Chem 2012; 287(19): 15672-83.
[http://dx.doi.org/10.1074/jbc.M111.322669] [PMID: 22431733]

[115] Elvira-Matelot E, Zhou X, Farman N, *et al.* Regulation of WNK1 expression by miR-192 and aldosterone. J Am Soc Nephrol 2010; 21(10): 1724-31.
[http://dx.doi.org/10.1681/ASN.2009111186] [PMID: 20813867]

[116] Luo Y, Liu Y, Liu M, *et al.* Sfmbt2 10th intron-hosted miR-466(a/e)-3p are important epigenetic regulators of Nfat5 signaling, osmoregulation and urine concentration in mice. Biochim Biophys Acta Gene Regul Mech 2014; 1839(2): 97-106.
[http://dx.doi.org/10.1016/j.bbagrm.2013.12.005] [PMID: 24389345]

[117] Orlowski S, Flees J, Anthony N, Dridi S. Differential expression of water channel- and noncoding RNA biogenesis-related genes in three lines of chickens under a short-term water restriction. Poult Sci 2017; 96(12): 4172-81.
[http://dx.doi.org/10.3382/ps/pex263] [PMID: 29053842]

[118] Jourjine N, Mullaney BC, Mann K, Scott K. Coupled Sensing of Hunger and Thirst Signals Balances Sugar and Water Consumption. Cell 2016; 166(4): 855-66.
[http://dx.doi.org/10.1016/j.cell.2016.06.046] [PMID: 27477513]

[119] Alhadeff AL, Su Z, Hernandez E, *et al.* A Neural Circuit for the Suppression of Pain by a Competing Need State. Cell 2018; 173(1): 140-152.e15.
[http://dx.doi.org/10.1016/j.cell.2018.02.057] [PMID: 29570993]

[120] Burnett CJ, Li C, Webber E, *et al.* Hunger-Driven Motivational State Competition. Neuron 2016; 92(1): 187-201.
[http://dx.doi.org/10.1016/j.neuron.2016.08.032] [PMID: 27693254]

[121] Padilla SL, Qiu J, Soden ME, *et al.* Agouti-related peptide neural circuits mediate adaptive behaviors

in the starved state. Nat Neurosci 2016; 19(5): 734-41.
[http://dx.doi.org/10.1038/nn.4274] [PMID: 27019015]

[122] Takei Y, Bartolo RC, Fujihara H, Ueta Y, Donald JA. Water deprivation induces appetite and alters metabolic strategy in *Notomys alexis* : unique mechanisms for water production in the desert. Proc Biol Sci 2012; 279(1738): 2599-608.
[http://dx.doi.org/10.1098/rspb.2011.2627] [PMID: 22398167]

# CHAPTER 7

# Proteins, Amino Acids, and Nitrogen Metabolism

**Abstract:** Animals require proteins, which are large nutrients made up of amino acids, in their diets. The body can make some amino acids, but others (essential amino acids) need to be provided by the diet. The ingested proteins go through digestion *via* various digestive enzymes to produce amino acids and peptides that are absorbed and transported *via* specific transporters. The whole body protein pool is determined by the balance between the processes of protein synthesis and degradation, which are under the control of hormonal, nutritional, and neuronal factors. Protein and amino acids play crucial roles in cellular and body weight homeostasis from regulation of appetite and food intake, metabolic reactions to cellular signaling within and between cells as well as energy production for survival. This chapter aims to discuss protein and amino acid metabolisms and provide a summary of current progress in the field.

**Keywords:** Amino acids, Absorption, Autophagy, Digestion, Protein synthesis, Protein degradation, Proteins, Ubiqitine-proteasome.

## INTRODUCTION

As described in chapter 1, an adult eats approximately a ton of food/year, which contains substrates (proteins, lipids, carbohydrates, vitamins, minerals, water) that provide the body with the necessary energy (metabolic fuels) for surviving, maintenance, growth, and reproduction. After describing the regulation of water and food intake in the previous chapters, I will describe here protein digestion, absorption, and metabolism.

### 7.1. Protein and Amino Acid Structure

The word protein is derived from the Greek word “proteos”, which means “primary” or “to take place first”. Protein was discovered in the early 19$^{th}$ century and was described as a nitrogen-containing part of food essential to life. Compared to other macronutrients that contain only carbon, oxygen, and hydrogen, proteins contain also nitrogen (N) and sulfur (S).

Proteins are polypeptides-polymers of amino acids linked by peptide bonds. Although approximately 140 types or more of amino acids are known to exist in

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nature, plant and animal proteins are composed of about 20 amino acids arranged in diverse sequences to form specific proteins. The biological properties of a protein are, therefore, determined by the amino acids it contains, the sequence in which they are linked together, and the configuration and spatial relationships among amino acids within the protein molecule.

All amino acids contain at least one amino group ($-NH_2$) and one carboxyl group (-COOH) (Fig. **7.1a-d**), with the exception of proline which is an imino acid as it lacks a free amino group. A common characteristic of amino acids found in proteins is that they have an asymmetric or alpha carbon, and thereby called alpha amino acid and constitute the major building block of proteins. In addition to the NH2 and COOH groups, the alpha carbon is attached to hydrogen and one additional group designated as R-group or side group that can vary in size and length. The R group is unique from one amino acid to the next (Fig. **7.1b**). For example, in glycine, R is a hydrogen atom; in alanine, it is a methyl group (-CH3), while in methionine, it is –CH2-CH2-S-CH3.

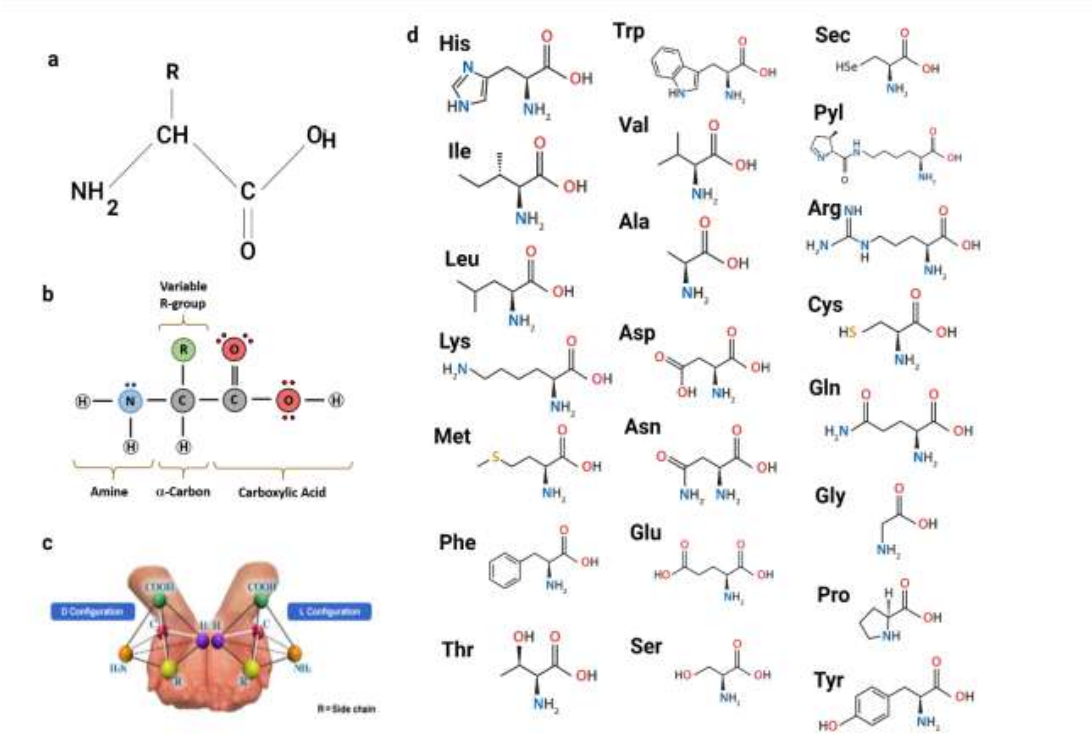



**Fig. (7.1). Amino acid structure. a)** General structure; **b)** Alpha amino acid, **c)** L- and D-isomere structure, and **d)** structure of all amino acids. Figures a, b, and d were made using Biorender. Figure c was adapted with permission from Ajinomoto.com.

Amino acids can exist in two isomeric forms, namely the D- and L-isomers. The two isomers differ in their configuration of groups around the asymmetric alpha-carbon. D-isomers (dextrorotary) rotate the plane of polarized light to the right however, L-isomers (levorotary) rotate it to the left (Fig. **7.1c**). Only the L-amino acids are used in protein synthesis. Some amino acids such as methionine can be converted by animals from D- to L-isomers, and that is why DL-methionine (racemic mixture) is used as a feed additive. However, the animal cannot convert, for example, the D-lysine, and thus commercial lysine is available only as L-lysine.

## 7.2. Classification of Amino Acids

Amino acids can be classified into three categories: essential or indispensable, non-essential or dispensable, and conditionally essential (Table **7.1**). Essential amino acids (phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine) cannot be synthesized by the organism and must therefore be produced by the diet. Non-essential amino acids (alanine, aspartic acid, asparagine, glutamic acid, serine, and selenocysteine, which are considered the 21$^{st}$ amino acid) can be synthesized in sufficient quantities in the body. An additional non-essential amino acid, pyrrolysine, is not used for most organisms, including humans. The synthesis of conditionally essential amino acids, however, can be limited by special (patho)physiological conditions, and these amino acids are arginine, cysteine, glycine, glutamine, proline, and tyrosine.

**Table 7.1. Essential, conditionally essential, and non-essential amino acids.**

| Essential amino acids | Non-essential amino acids | Conditionally essential amino acids |
|---|---|---|
| Histidine (His or H) | Alanine (Ala or A) | Arginine (Arg or R) |
| Isoleucine (Ile or I) | Aspartic acid (Asp or D) | Cysteine (Cys or C) |
| Leucine (Leu or L) | Asparagine (Asn or N) | Glutamine (Gln or Q) |
| Lysine (Lys or L) | Glutamic acid (Glu or E) | Glycine (Gly or G) |
| Methionine (Met or M) | Serine (Ser or S) | Proline (Pro or P) |
| Phenylalanine (Phe or F) | Selenocysteine (Sec or U) | Tyrosine (Tyr or Y) |
| Threonine (Thr or T) | Pyrrolysine (Pyl or O) | |
| Tryptophan (Trp or W) | | |
| Valine (Val or V) | | |

Additionally, and based on the characteristics of the R group and the nature of atoms incorporated in the functional groups, amino acids can be further categorized as polar, non-polar, acidic, basic, and also to aliphatic, aromatic, and positively or negatively charged (Table **7.2**).

**Table 7.2. Classification of amino acids.**

| **Hydrophobic Amino Acids - Nonpolar** | | **Hydrophilic Amino Acids - Polar** | | |
|---|---|---|---|---|
| **Alkyl Side Chain** | **Aromatic Side Chain** | **Neutral** | **Acidic** | **Basic** |
| Glycine | Phenylalanine | Tyrosine | Glutamic Acid | Lysine |
| Alanine | Tryptophan | Serine | Aspartic Acid | Histidine |
| Valine | - | Threonine | - | Arginine |
| Leucine | - | Cysteine | - | - |
| Isoleucine | - | Glutamine | - | |
| Methionine | - | Asparagine | - | - |
| Proline | - | - | - | - |

Polar amino acids are those whose side chains are capable of forming one or more hydrogen bonds, have a polarity, and are hydrophilic. These amino acids can be neutral, positively, or negatively charged (Table **7.2**). Depending on the pK and the pH of a solution, the polar amino acids with a positive charge contain more NH2 group than COOH groups and are therefore basic. When the polar amino acid (side chain) loses a proton, they are negatively charged and they are therefore acid. The nonpolar amino acids can largely be subdivided into two more specific classes, the aliphatic and the aromatic amino acids. The aliphatic amino acids typically contain branched hydrocarbon chains, with the simplest being glycine to the more complicated structures of leucine and valine. The aromatic amino acids contain aromatic functional groups within their structure, making them largely nonpolar and hydrophobic due to the high carbon/hydrogen content (Table **7.2**).

Amino acids are also classified as standard and non-standard amino acids. The standard ones are those encoded by universal genetic codes and are incorporated in protein sequences. The Non-standard amino acids, however, are incorporated into proteins by a unique synthetic mechanism. Selenocysteine, for example, is incorporated when mRNA translated included selenocysteine insertion sequence (SECIS), which causes the UGA codon to encode for selenocysteine instead of the stop codon. Pyrrolysine, on the other hand, is an α-amino acid encoded by the stop codon UAG and is involved in protein synthesis in some methanogenic archaea and bacteria [1]. Other classifications of amino acids include: 1) non-protein amino acids, which are those amino acids found in the free state as intermediates of metabolic pathways for standard amino acids and they are not encoded by genetic codes such as ornithine and citrulline involved in urea biosynthesis. 2) Non α-amino acids where the NH2 group is not attached to the α-carbon atom such as γ-aminobutyric acid (GABA) and β-alanine [2]. 3) Modified protein-amino acids, which are amino acids that undergo modification such as

hydroxylation of proline and lysine to form hydroxyproline and hydroxylysine, respectively, and are essential for mature collagen formation [3 - 5].

Among the 20 amino acids, there are three branched-chain amino acids (BCAAs), leucine, isoleucine, and valine, and are so-called because of their structures include a branched side chain with carbon and hydrogen atoms (Fig. **7.2**). These BCAAs play many key physiological roles such as protein synthesis and energy production. The requirements of BCAAs and essential amino acids by age categories is summarized in Table **7.3**.

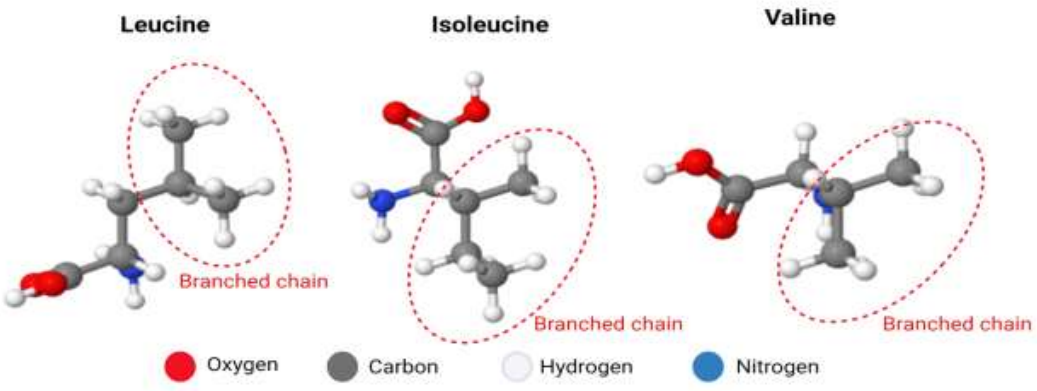



**Fig. (7.2).** Structure of branched-chain amino acids (BCAAs).

**Table 7.3. Requirement in mg/kg BW/day of essential amino acids by age categories.**

| - | - | Age Category | |
|---|---|---|---|
| **Essential Amino Acids** | **2 Years** | **10-12 Years** | **Adult** |
| Leucine | 73 | 45 | 14 |
| Isoleucine | 37 | 30 | 10 |
| Valine | 38 | 33 | 10 |
| Tryptophan | 12.5 | 4 | 3.5 |
| Threonine | 37 | 35 | 7 |
| Lysine | 64 | 60 | 12 |
| Methionine and Cysteine | 27 | 27 | 13 |
| Phenylalanine and tyrosine | 69 | 27 | 14 |

To form a (poly) peptide or proteins, these amino acids are linked together by peptide bonds. This bond is formed by the reaction of the α-amino group of one amino acid with the α-carboxyl group of another amino acid. A dipeptide contains two amino acids linked by one peptide bond. If an additional amino acid is added,

a tripeptide is formed. A polypeptide contains more than ten amino acids. A protein is a large polypeptide with a molecular weight expressed in Dalton, the mass of hydrogen ions.

## 7.3. Protein Digestion and Absorption

Protein digestion is the breakdown of protein into end products (single amino acids) *via* hydrolysis of the peptide bonds between amino acids. It is a complex process in that a variety of enzymes and tissues are involved (Fig. **7.3**). The protein digestion starts in the stomach with a secretion of fluid rich in HCl (pH 0.8-0.9) by stomach parietal cells[1], which creates a very acidic milieu (pH 1.5-2.5) when mixed with other stomach intrinsic factors of the gastric juice. This stomach acidic environment induces the secretion and the activation of pepsin from pepsinogen in the chief mucosal cells[2] and enhances its proteolytic activity. In addition, the acidity of the stomach denatures proteins, which in turn facilitates the access of proteolytic enzymes. Activated pepsin stimulates hydrolysis at peptide bonds involving the carboxyl group of the aromatic amino acids and probably acidic amino acids. The hydrolyzed proteins from the stomach enter the small intestine along with the pancreatic inactive zymogens trypsinogen, chymotrypsinogen, and procarboxypeptidase A/B. The intestinal mucosal cells secrete the enterokinase enzyme that cleaves trypsinogen into active trypsin, which in turn cleaves further the trypsinogen to form more trypsin. Trypsin acts as an endopeptidase to cleave proteins and peptides internal to the chain as well as the peptide linkages involving the carboxyl groups of arginine and lysine. Trypsin also activates the other zymogens to form chymotrypsin and carboxypeptidases A and B. Chymotrypsin produces small polypeptides, peptides, and some individual amino acid *via* degradation of proteases, denatured proteins, peptones, and large polypeptides. Chymotrypsin is an endopeptidase specific for bonds involving the carboxyl ends of phenylalanine, tyrosine, and tryptophan. Carboxypeptidase A cleaves the carboxyl terminal residues that possess aromatic and aliphatic linkage. Carboxypeptidase B hydrolyzes the terminal residues of arginine and lysine. In order to be protected from auto-digestion, the pancreas produces intrinsic trypsin inhibitors.

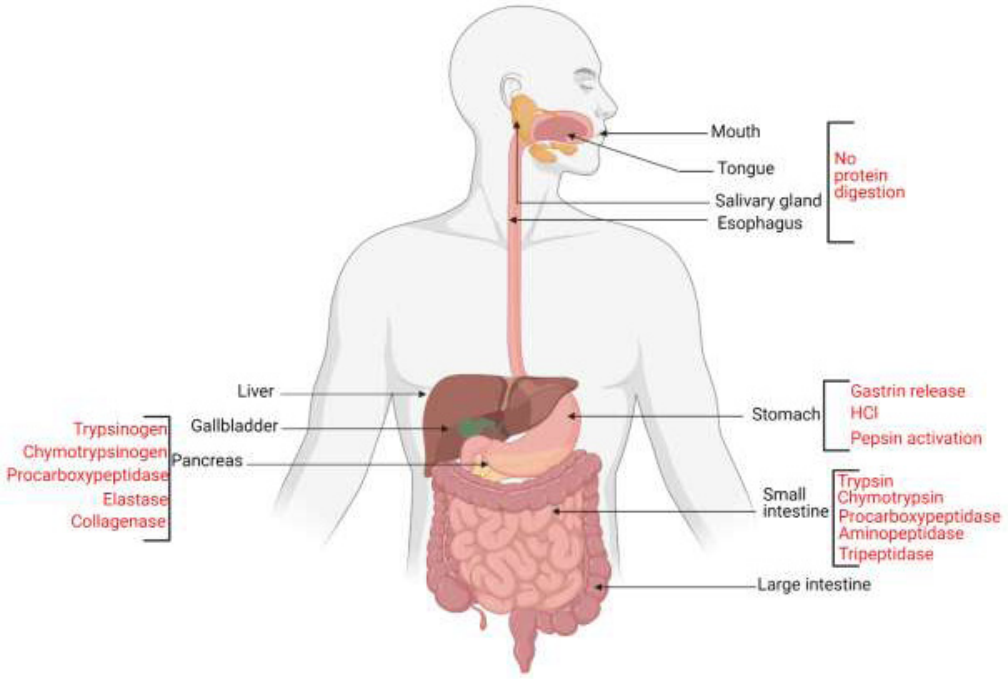



**Fig. (7.3).** Protein digestion: tissues and enzymes involved.

These peptides produced by the gastric and pancreatic reactions (enzymes) are subjected to the final step of digestion and hydrolysis in microvilli membranes of the intestinal mucosal cells, where aminopeptidases, tripeptidases, and dipeptidases produce short oligopeptide fragments, dipeptides and single amino acids.

The end products of the digestion and the action of endopeptidases and exopeptidases are a mixture of free amino acids, dipeptides, tripeptides, and short oligopeptides, all of which are absorbed from the intestinal lumen and then hydrolyzed to single amino acids by intracellular peptidases. The oligopeptides (dipeptides and tripeptides), major end-products of protein digestion, enter by $H^+$-coupled oligopeptide transporter SLC15A1 (PepT1), and once inside of the enterocytes, the oligopeptides are degraded by di- and tri-peptidases into single amino acids (Fig. **7.4**). Free amino acids use $Na^+$- or $H^+$-dependent active transport. The inwardly directed $Na^+$ electrochemical gradient that is used as a driving force for the uptake of $Na^+$-coupled amino acids transport is maintained by the basolateral $Na^+/K^+$-ATPase. To enable $H^+$-coupled amino acid uptake, the $Na^+$ gradient is converted to an inwardly directed $H^+$ gradient by the apical SLC9A3 (NHE3) $Na^+/H^+$ exchanger. Amino acid transporters are membrane-bound transport proteins that mediate the transfer of amino acids into and out of cells or

organelles. There are several different amino acid transporters, with specificity for the nature of the side chain of the amino acid, including aromatic, cationic, and neutral amino acids (Table **7.4**). On the basolateral side, several amino acid transporters facilitate the exit of various amino acids into the circulation (blood) (Fig. **7.4**).

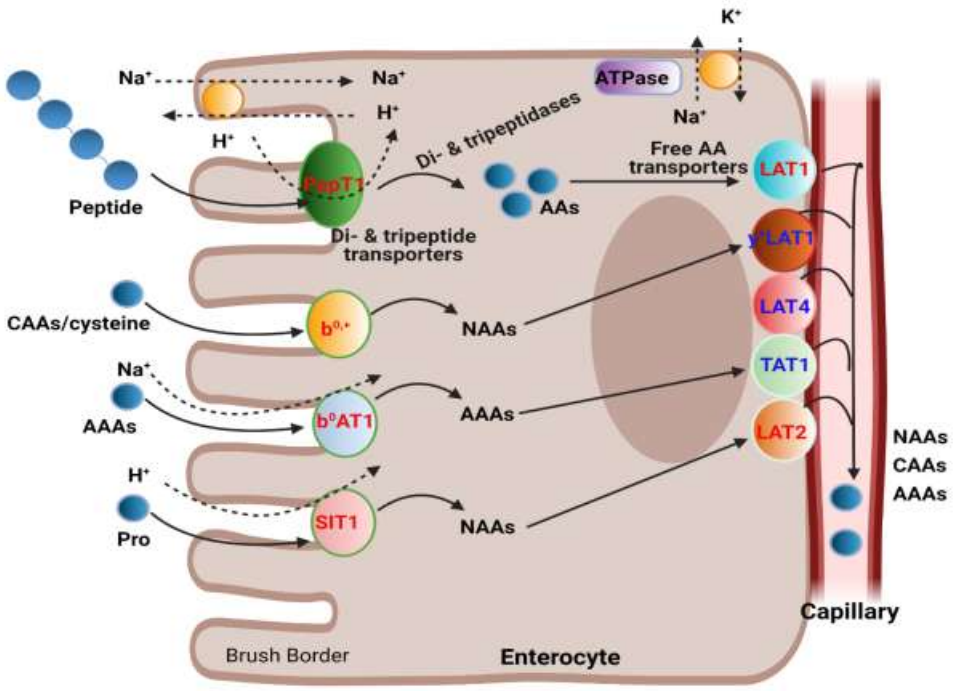



**Fig. (7.4). Major intestinal amino acid transporters.** Representative illustration showing major amino acid transporters involved in intestinal amino acid absorption. The oligopeptides (dipeptides and tripeptides), major end-products of protein digestion, enter by $H^+$-coupled oligopeptide transporter SLC15A1 (PepT1), and once inside of the enterocytes, the oligopeptides are degraded by di- and tri-peptidases into single amino acids. Free amino acids use $Na^+$- or $H^+$-dependent active transport. The inwardly directed $Na^+$ electrochemical gradient that is used as a driving force for the uptake of $Na^+$-coupled amino acids transport is maintained by the basolateral $Na^+/K^+$-ATPase. To enable $H^+$-coupled amino acid uptake, the $Na^+$ gradient is converted to an inwardly directed $H^+$ gradient by the apical SLC9A3 (NHE3) $Na^+/H^+$ exchanger. AA, amino acid; AAA, aromatic AA; CAA, cationic AA; NAA, neutral AA; Pro, proline.

**Table 7.4. Intestinal amino acid transporters.**

| Protein Name | Gene Name | System | Substrates |
|---|---|---|---|
| EAAT3/EAAC1 | SLC1A1 | $X^-$ AG/$Na^+$, $H^+$, $K^+$ | E, D, C |
| ASCT1 | SLCA4 | ASC/antiporter | A, S |
| ASCT2 | SLC1A5 | ASC/antiporter | D, C, Q |
| $B^0$AT1 | SLC6A19 | $Na^+$ | NAAs |

*(Table 4) cont.....*

| Protein Name | Gene Name | System | Substrates |
|---|---|---|---|
| SIT1/IMINO | SLC6A20 | $Na^+/Cl^-$ | P |
| CAT-1 | SLC7A1 | $y^+$/uniporter | CAAs (R) |
| $y^+$LAT1/LAT2 | SLC7A6/A7 | $y^+L$ | CAAs, LNAAs |
| LAT2 | SLC7A8 | L/antiporter | LNAAs |
| $b^{0,+}$AT | SLC7A9 | $b^{0,+}$ | CAAs, NAAs, C |
| Asc-1 | SLC7A10 | Asc/antiporter | Small NAAs |
| PHT2 and PHT1 | SLC15A3/A4 | $H^+$ | H |
| MCT10/TAT1 | SLC16A10 | Facilitated transporter | AAAs (F, Y, W) |
| ORC2/ORC1 | SLC25A2/A15 | $H^+$/antiporter | R, H, Orn, Cit |
| PAT1 | SLC36A2 | $H^+$ | GABA |
| LAT4 | SLC43A2 | L | BCAAs |

A, alanine; AAA, aromatic amino acid; ASCT, alanine, serine, cysteine, and threonine exchanger; $b^0$AT1, system B(0) neutral amino acid transporter; BCAA, branched-chain amino acid; C, cysteine; CAA, cationic amino acid; CAT, cationic amino acid transporter; Cit, citrulline; D, aspartic acid; E, glutamic acid; EAAT, excitatory amino acid transporter; GABA, gamma-aminobutyric acid; H, histidine; LAT, $Na^+$-independent system L neutral amino acid transporter; LNAA, large neutral amino acid; MCT10/TAT, monocarboxylate transporter 10/T-type amino acid transporter; NAA, neutral amino acids; ORC, mitochondrial ornithine transporter; P, proline; Orn, ornithine; PAT, proton-dependent amino acid transporter; PHT, peptide/histidine transporter; Q, glutamine; R, arginine; S, serine; SIT, sodium/imino acid transporter; $y^+$LAT, $y^+$L amino acid transporter.

Based on their transport function, amino acid transporters are classified in different systems (A, N, ASC, L, T, $x^-_c$, and $y^+$). These systems have acronyms that denote substrate specificity. Uppercase letters indicate $Na^+$-dependent transporters, with the exception of L and T systems. Lowercase letters denote $Na^+$-independent transporters. $x^-$ indicates transporter for AAA. In $X^-_{AG}$, AG indicates that the transporter accepts aspartate and glutamate. In $x^-_c$, the subscript c indicates that the transporter accepts cysteine. $y^+$ denotes transporters for cationic amino acids. B refers to a transporter accepting NAAs, and superscript + indicates a transporter for CAA. T indicates transporter for AAA, N indicates transporter selective for an amino acid with N atoms in the side chain, L system indicates a preference to leucine as substrate, and ASC indicates a transporter preferring alanine.

## 7.4. Protein Synthesis

### *7.4.1. Importance of Balanced Dietary Amino Acids*

Ingested proteins need to be of high quality and meet the body's needs for growth, survival, immunity, energy homeostasis, and daily functions. A protein that

contains the required essential amino acids will be completely useable for tissue protein synthesis however a protein deficient in one or more of the essential amino acids will not. Similarly excess essential amino acids can be detrimental. For instance, as described in previous chapters, excess leucine was shown to depress feed intake and growth [6]. On the other hand, histidine appears to be one of the most toxic amino acids, and high dietary histidine has been shown to cause serious adverse effects in both humans and animals [7]. In animals, excess dietary lysine leads to reduced growth and feed intake without any apparent toxicity but induces accumulation of triglycerides in the liver [8, 9]. In humans, lysine excess is toxic and was used as a treatment for the herpes virus [10]. Excess supplementation of methionine suppressed feed intake, stopped growth, induced hemosiderin accumulation and erythrocyte engorgement, as well as liver damage in animals [11 - 13]. High dietary tryptophan levels reduce feed intake depressed motor activity and affect sleep latency in animals [14]. In humans, tryptophan supplementation was associated with the outbreak of eosinophilia-myalgia syndrome (EMS)[3] in the eighty [15]. Concerns of phenylalanine safety arose from the brain development abnormality observed in humans with phenylketonuria and in rodents administered with phenylalanine [16]. Excess of dietary non-essential amino acids can be harmful also. For example, rats on low-protein diets died following intake of high tyrosine levels. Similarly, high plasma tyrosine levels were associated with eye lesions and mental retardation [17]. Together, the intensive nutritional studies, the bulk of data, and the rich literature indicate supplementation and dosage of amino acids need to be closely managed because insufficiency or excess can be detrimental to cell growth and metabolism, including defective cellular protein synthesis.

### *7.4.2. Mechanism of Protein Synthesis*

#### *7.4.2.1. Transcription*

The blueprint for protein synthesis in most organisms is the nucleotide sequence of the DNA that carries the raw genetic information, which can be turned into functional products, usually proteins. Protein synthesis starts with the production of a strand of messenger RNA that is complementary to the DNA gene being expressed in a process called transcription[4]. Transcription is carried out by an enzyme called RNA polymerase and a number of accessory proteins called transcription factors, activators, and repressors. To initiate transcription, RNA polymerase recognizes the promoter region at the beginning of the gene (Fig. **7.5**), open the double-strand DNA, and initiates the synthesis and the elongation of mRNA. Three RNA polymerases were characterized: Pol I produces the large ribosomal RNA precursor, Pol II produces mRNAs and various non-coding RNAs, and Pol III produces transfer RNAs and small ribosomal RNAs.

After the opening of the chromatin, Pol II gains access to the promoter region of the target expressed gene [18]. Different classes of promoters are known, some contain CpG islands which are found in housekeeping genes [19]. Other promoters contain TATA elements upstream of the transcription start site [20]. The activation of the promoters is under the control of nuclear transcription factors that bind, in a sequence-dependent manner (called response element), to DNA and guide polymerase to their targets [21 - 24]. Approximately 1,600 transcription factors are known in humans. These transcription factors can recruit proteins, including histone acetyltransferases that regulate promoter accessibility and initiation of transcription. The first step is the recognition of the promoter where the Pol II assembles with transcription initiation factors such as TBP and TFIIB to form a specific pre-initiation complex (PIC) on the promoter and induces initial mRNA synthesis [25 - 27] (Fig. **7.5**).

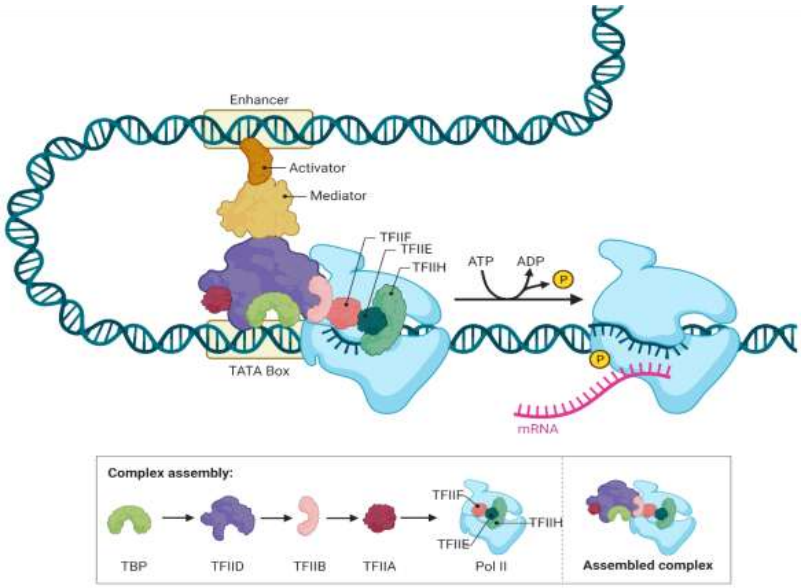



**Fig. (7.5). Protein synthesis mechanism: transcription cycle.** Pol II assembles with transcription initiation factors such as TBP and TFIIB to form a specific pre-initiation complex (PIC) on the promoter and induces initial mRNA synthesis. The transcription initiation is regulated by the mediator, which facilitates the transition to the phase of transcription elongation. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Pol II, RNA polymerase II; TBP, TATA-box binding protein; TF, transcription factor.

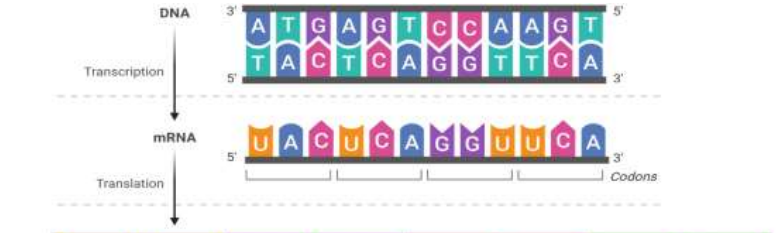
Next, Pol II uses XPB, DNA translocase that hydrolyses ATP to unwind DNA and open the promoter [28]. Transcription initiation is regulated by co-activator complex, also known as mediator [29] that stimulates the phosphorylation of Pol II and facilitates the transition to the phase of transcription elongation. When the RNA strand reaches a critical length, the elongation complex (DNA-RNA hybrid)

is formed [30] and Pol II moves from one template position to another by closing the active site and catalyzing the formation of a phosphodiester bond [31]. The recruitment of the capping enzyme results in the protection of the nascent RNA 5' end with a cap structure [32] and the mature single-strand RNA is transported to the cytoplasm (ribosome) for translation.

### *7.4.2.2. Translation*

Translation is the second major step in gene expression and protein synthesis. It is simply a reading and decoding process of mRNA to produce a specific sequence of amino acids and to synthesize proteins, which are the workhorses of the living cell. These proteins (enzymes, housekeeping, *etc*.) carry out all the functions necessary for life.

Three key components, namely mRNA, ribosome, and transfer RNA are required during the translation. The nucleotide bases of mRNA are read as codons of three bases, and each codon specifies a unique amino acid (Fig. **7.6**). Multiple codons can code for the same amino acids, such as AGA, CGC, and AGG for arginine.




| | U | C | A | G |
|---|---|---|---|---|
| U | UUU = phe | UCU = ser | UAU = tyr | UGU = cys |
| | UUC = phe | UCC = ser | UAC = tyr | UGC = cys |
| | UUA = leu | UCA = ser | UAA = stop | UGA = stop |
| | UUG = leu | UCG = ser | UAG = stop | UGG = trp |
| C | CUU = leu | CCU = pro | CAU = his | CGU = arg |
| | CUC = leu | CCC = pro | CAC = his | CGC = arg |
| | CUA = leu | CCA = pro | CAA = gln | CGA = arg |
| | CUG = leu | CCG = pro | CAG = gln | CGG = arg |
| A | AUU = ile | ACU = thr | AAU = asn | AGU = ser |
| | AUC = ile | ACC = thr | AAC = asn | AGC = ser |
| | AUA = ile | ACA = thr | AAA = lys | AGA = arg |
| | AUG = met | ACG = thr | AAG = lys | AGG = arg |
| G | GUU = val | GCU = ala | GAU = asp | GGU = gly |
| | GUC = val | GCC = ala | GAC = asp | GGC = gly |
| | GUA = val | GCA = ala | GAA = glu | GGA = gly |
| | GUG = val | GCG = ala | GAG = glu | GGG = gly |

**Fig. (7.6). mRNA codons.** The nucleotide bases of mRNA are read as codons of three bases, and each codon specifies a unique amino acid. Multiple codons can code for the same amino acids. The codons are written 5' to 3' as they appear in the mRNA sequence. AUG code for the start codon "methionine", and UAA, UAG, and UGA are stop codons.

The translation of the coding region, but not that of the untranslated region (UTR), starts at the 5' cap of the mRNA. The first step in the translation process is the initiation step with the formation of the pre-initiation complex containing initiation factor proteins (IF1, 2, and 3), ribosome small subunits, and the Met-carrying tRNA. Subsequently, the ribosome's large subunit binds to complete the initiation complex. The ribosome's large subunit binds to the initiation complex. The ribosome's large subunit has three binding sites: an amino acid site A, a polypeptide site P, and an exit site E. The Met-tRNA (carrying methionine) binds to the start codon AUG of the mRNA at the ribosome's P site where it will be the first amino acid incorporated in the protein chain at the N-terminal side. Methionine moves from the P site to the A site on the ribosome's large subunit to bond to a new amino acid there, and so the growth of the peptide has begun. The tRNA molecule in the P site no longer has attached amino acid and thereby leaves the ribosome. The ribosome translocates along the mRNA to the next codon and the polypeptide chain is built up in the direction from N- to C-terminal (Fig. **7.7**). At the terminal step, one of the three stop codons (UAA, UAG, or UGA) enters the ribosome A site to block any tRNA binding, and then the peptide and tRNA in the P site become hydrolyzed, releasing the polypeptide into the cytoplasm.

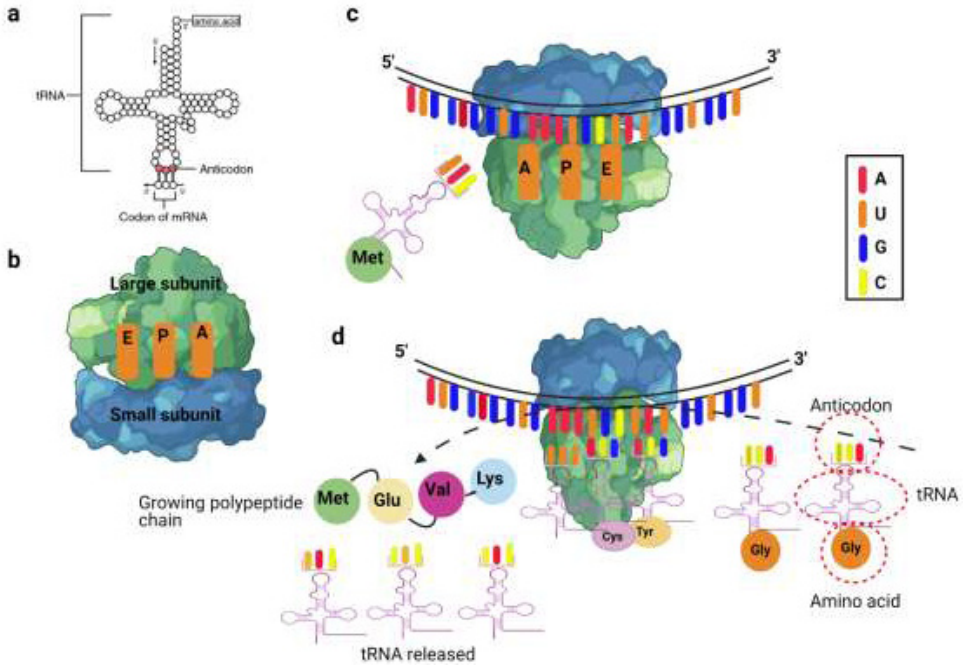



**Fig. (7.7). Translation of protein synthesis.** The translation process incorporates 20 different amino acids in the presence sequence dictated by the three-base codons. **a)** tRNA structure, **b)** rRNA structure, **C)** translation initiation, and **d)** translation elongation and termination.

Protein synthesis is an energy-expensive process. Energy is needed for peptide bond formation (4 mol of ATP equivalent per each peptide bond), attachment of amino acid to tRNA (2 mol of ATP per each amino acid attached), attachment of tRNA to ribosome A site, and the movement of the growing polypeptide from the A to the P site.

### *7.4.2.3. Regulation of Protein Synthesis by Mechanistic Target of Rapamycin (mTOR)*

Several extrinsic factors regulate protein synthesis. mTOR, which controls a number of translation initiation and elongation, is the most studied factor (Fig. **7.8**). In fact, protein synthesis induced by insulin, growth factors, or other growth-promoting agonists is inhibited by rapamycin. The downstream cascade of mTOR includes activation of the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and the S6 kinases. The eIFs mediate key steps in translation initiation, such as the recruitment of the mRNA to the small ribosome subunit and the recruitment of Met-tRNA. Furthermore, stimuli (insulin, amino acids) that activate mTOR induce translation elongation. Blockade mTOR by AMPK slows elongation [33]. The S6 kinase lies in a region of the ribosome close to the interface between the two ribosome subunits (small and large), and contacts mRNA and tRNA. Rapamycin inhibits S6 Kinase phosphorylation and protein synthesis [34]. mTOR regulates the ribosome biogenesis and the transcription of polymerase RNAs [35 - 38].

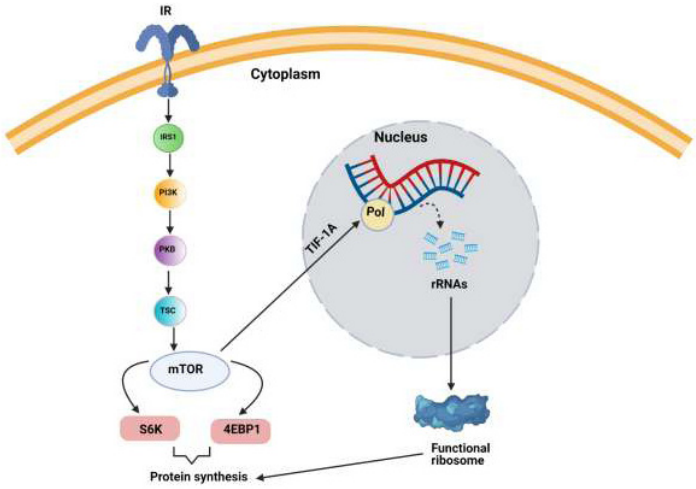



**Fig. (7.8). Role of mTOR in protein synthesis.** A schematic depiction of regulation of protein synthesis by mTOR. Activation of mTOR by insulin and growth factors results in activation of the eukaryotic translation initiation factor 4E-binding proteins and the ribosomal S6 kinases, which in turn induce protein synthesis. mTOR also regulates polymerase and rRNA biogenesis. IR, insulin receptor; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; TIF-1, tripartie motif-containing protein 1; TSC, tuberous sclerosis protein.

### *7.4.2.4. Protein Structure*

The primary structure of a protein is simply the sequence of the linked amino acids that are placed in a very specific order dictated by genetic information. This primary protein sequence is linked together by peptide bonds formed by dehydration (water molecule extraction) reaction as it joins the amino group of one amino acid to the carboxyl group of the next neighboring amino acid. As polypeptide chains fold up in various ways and stabilize by hydrogen bonds, protein secondary structure is the three dimensional form of the polypeptide. The two most common patterns of protein secondary structure are alpha-helix. The peptide backbone of the protein adopts a spiral (helix) form *via* the formation of hydrogen bonds between close peptide bonds in the primary structure (Fig. **7.9**). Beta-pleated sheet, in which regions of the polypeptide chain lie alongside one another resulting in a pleated surface (Fig. **7.9**). Proteins can also form hairpins and omega loop shape, a non-regular protein structural motif consisting of a loop of six or more amino acid residues and any amino acid sequences. In addition, proteins can have a random coil in which there is no recognizable organized structure. A protein can have all of these shapes (several α-helix, β-pleated sheets, hairpins, and random coil) in the same molecule. The tertiary structure of a protein is the structure at which polypeptide chains become functional. It is the complete three-dimensional shape of the entire protein. In the quaternary structure, several subunits of a protein interact and stabilize the overall structure (Fig. **7.9**)

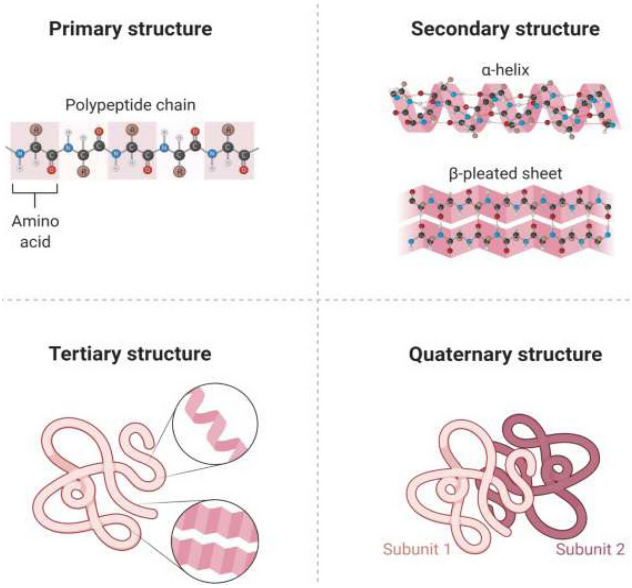



**Fig. (7.9).** Protein structures.

### *7.4.2.5. Protein and Amino Acid Modifications*

Many proteins are secreted hormones and contain signal peptides that need to be removed as part of the post-translational modifications (PTMs). Most of the newly synthesized proteins lose their initial amino-terminal methionine in a process called N-terminal methionine excision [39]. In addition, various proteins are associated and covalently bound to carbohydrates and lipids. Others are associated with vitamins, metal ions (metalloproteins), or heme prosthetic group (hemoproteins). Other PTMs occur, such as N-α-acetylation with a transfer of an acetyl moiety

from acetyl-CoA to the α-amine of the N-terminal amino acid of a nascent chain. Protein fatty-acylation is known as N-myristoylation, involves the irreversible transfer of a myristate moiety from myristoyl-CoA to the α-amino group of glycine of the target protein. Precursor proteins also undergo maturation and pre peptide cleavage at the N- or C-terminal signal peptide (mitochondrial- or peroxisome- terminal signal peptide) for selective sorting to target subcellular compartments. As mentioned above, proteins can covalently link to sugars in a process known by glycosylation [40]. Protein phosphorylation, the most widespread PTMs and the most extensively studied, plays a key role in protein activity, subcellular localization, and signaling cascades *via* the action of protein kinases that transfer a phosphate from ATP to the protein [41, 42]. Proteins can also undergo oxidation (carbonylation, sulfhydryl oxidation, *etc*.), ubiquitination, SUMOylation, disulphide bonding, and lipidation [43 - 46].

### *7.4.2.6. Amino Acid Metabolism*

In terms of energy sources, amino acids are different from other macronutrients in that they contain nitrogen element. Thus, the removal or transfer of nitrogen is necessary for the utilization of amino acids as an energy source as well as the creation of nonessential amino acids and other molecules. The amino group (-NH3) of amino acids can be removed or transferred *via* deamination or transamination, respectively.

#### *7.4.2.6.1. Transamination*

Transamination reaction, *via* amino transferase enzyme, is the transfer of an amine group from an amino acid to α-keto acid (amino acid without an amine group), thus forming a new amino acid and an α-keto acid. Pyruvate and α-ketoglutarate and the two α-keto acids used most in transamination reactions (Fig. **7.10**).

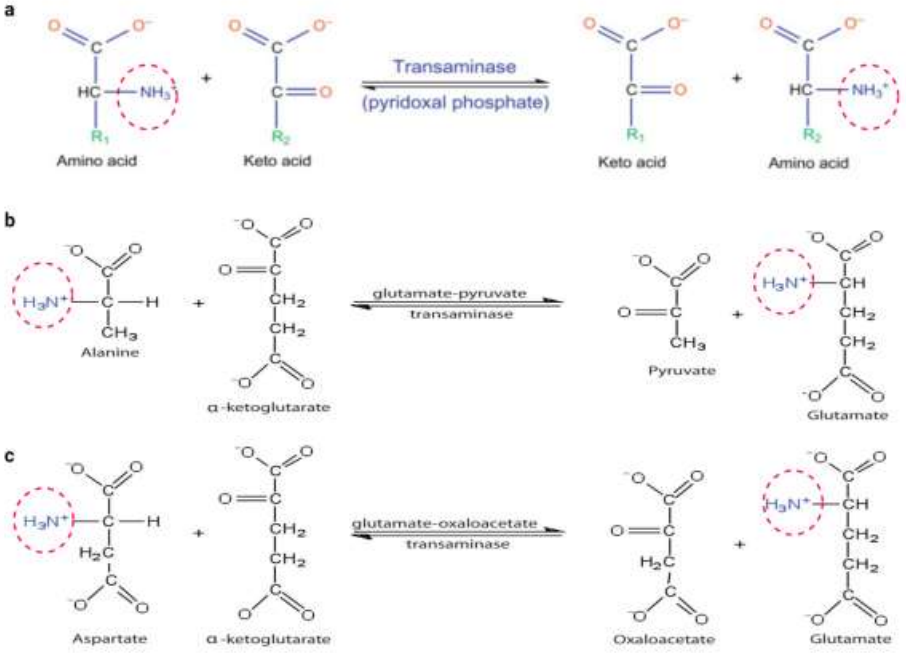



**Fig. (7.10). Transamination reaction. a)** General reaction, **b)** alanine transamination, and **c)** glutamate transamination.

## *7.4.2.6.2. Deamination*

Deamination reaction, which is catalyzed by deaminases, removes the amino group from amino acids to form ammonia (Fig. **7.11**). For example, oxidative deamination of glutamate by glutamate dehydrogenase produces α-ketoglutarate and free ammonia. Ammonia is quite toxic to animal tissues, and it is converted into a nontoxic compound before export from extrahepatic tissues

into the blood. In many tissues, ammonia is enzymatically combined with glutamate to yield glutamine by the action of glutamine synthetase, and glutamine (nontoxic, neutral compound) can readily pass through the cell membrane and is carried in the blood to the liver. In the liver, glutaminase converts glutamine to glutamate and ammonia (Fig. **7.11**). Glutamine is a major transport form of ammonia.

## *7.4.2.6.3. Fate of Amino Acid Degradation*

Once nitrogen is removed from amino acids, the rest carbon skeletal of amino acids are degraded to yield pyruvate, acetyl-CoA, Krebs cycle intermediates, and the ketone body acetoacetate (Fig. **7.12**). The amino acids whose carbon skeletons

from pyruvate or Krebs cycle intermediates are known as glucogenic amino acids because they can be used to produce glucose in the liver *via* gluconeogenesis pathway (see chapter 8, section 8.3.2.8). The amino acids whose carbon skeletons become acetyl-CoA and acetoacetate are known as ketogenic amino acids because they can form ketone bodies. Few amino acids (tryptophan, phenylalanine, threonine, tyrosine, and isoleucine) are both glucogenic and ketogenic.

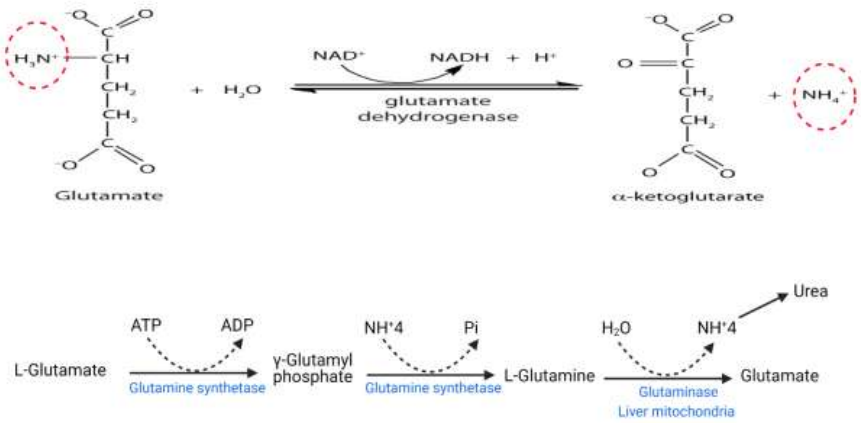



**Fig. (7.11).** Deamination reaction.

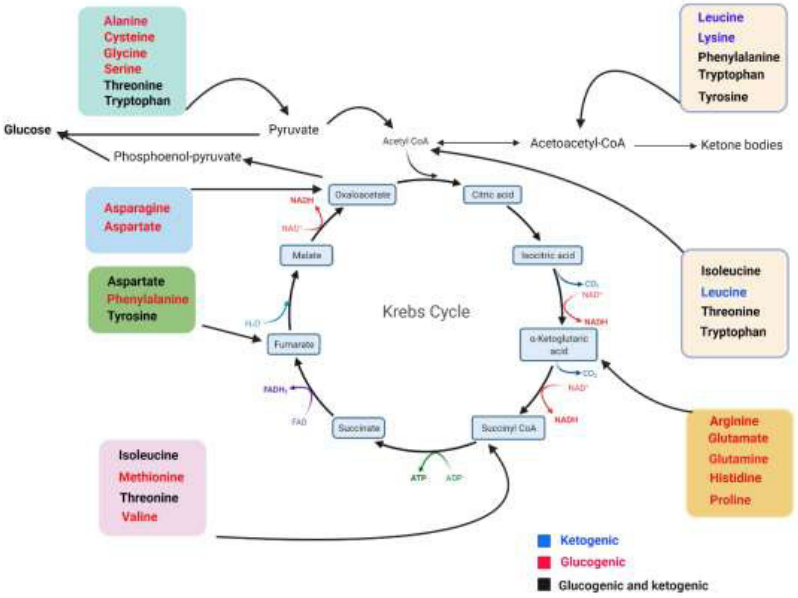



**Fig. (7.12). Amino acid catabolism.** Fate of glucogenic, ketogenic, and both glucogenic and ketogenic amino acids in the production of ketone bodies and Krebs cycle intermediates.

### *7.4.2.6.4. The Alanine-Glucose Cycle*

As for glutamine, alanine plays a crucial role in transporting an amino group to the liver in a nontoxic form by the glucose-alanine cycle. For example, during intensive exercise, skeletal muscle produces not only ammonia from protein breakdown but also a large amount of pyruvate from glycolysis (see chapter 8, section 8.3.2.1). These two products must reach the liver, where ammonia needs to be converted into urea for excretion, and pyruvate needs to be reused into glucose and returned to the muscle providing the necessary energy for contraction and function. For this end, animals use the alanine-glucose cycle to move the carbon atom of pyruvate and excess ammonia from muscle to liver (Fig. **7.13**). The muscle alanine passes into the circulation and travels to the liver. In hepatocyte cytosol, alanine aminotransferase transfers the amino group from alanine to α-ketoglutarate, yielding pyruvate and glutamate. Glutamate can undergo transamination with oxaloacetate to form aspartate (nitrogen donor in urea synthesis), or enter the mitochondria to produce α-ketoglutarate and ammonia for the urea cycle.

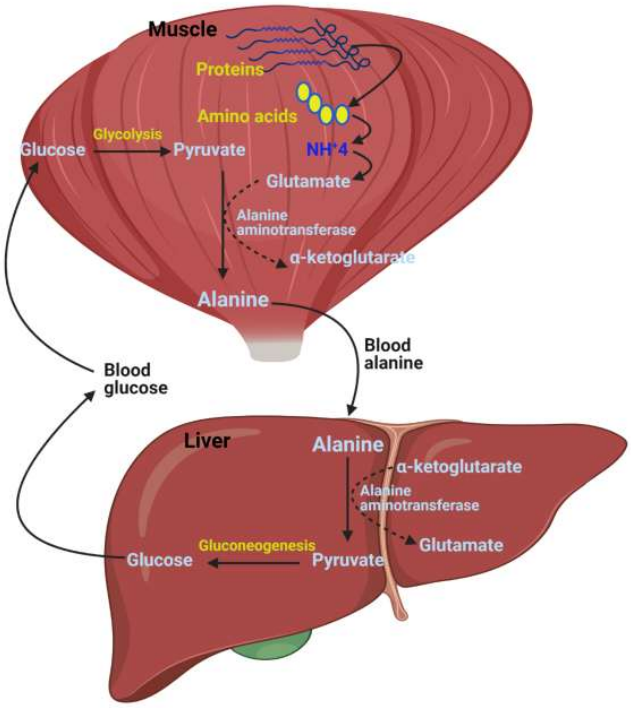



**Fig. (7.13). The alanine-glucose cycle.** It is also known as Cahill cycle, is a series of reactions in which the muscle alanine passes into the circulation and travels to the liver where pyruvate and glutamate are produced.

## *7.4.2.6.5. The Urea Cycle*

The urea cycle consists of five reactions that convert ammonia, carbon dioxide, and the α-amino nitrogen of aspartate into urea. Two of the reactions occur within the mitochondria, and the rest of the reactions occur in the cytoplasm (Fig. **7.14**). Carbon dioxide in the mitochondria is phosphorylated through ATP and condensed to ammonia. Catalyzed by carbamoyl phosphate synthase, $CO_2$ and $NH_4^+$ form the cabamoyl phosphate. Carbamoyl phosphate is condensed with ornithine in the mitochondria *via* ornithine transcarbamoylase to form citrulline. Citrulline enters the cytoplasm and condenses with aspartate *via* arginosuccinate synthetase to form arginosuccinate, which is subsequently split by argininosuccinase into fumarate and arginine. Arginine is then hydrolyzed by arginase to produce urea and ornithine. In chickens (*Gallus gallus*), little urea is synthesized due to the absence of carbamyl phosphate synthetase and the N-acetyl glutamate synthase [47].

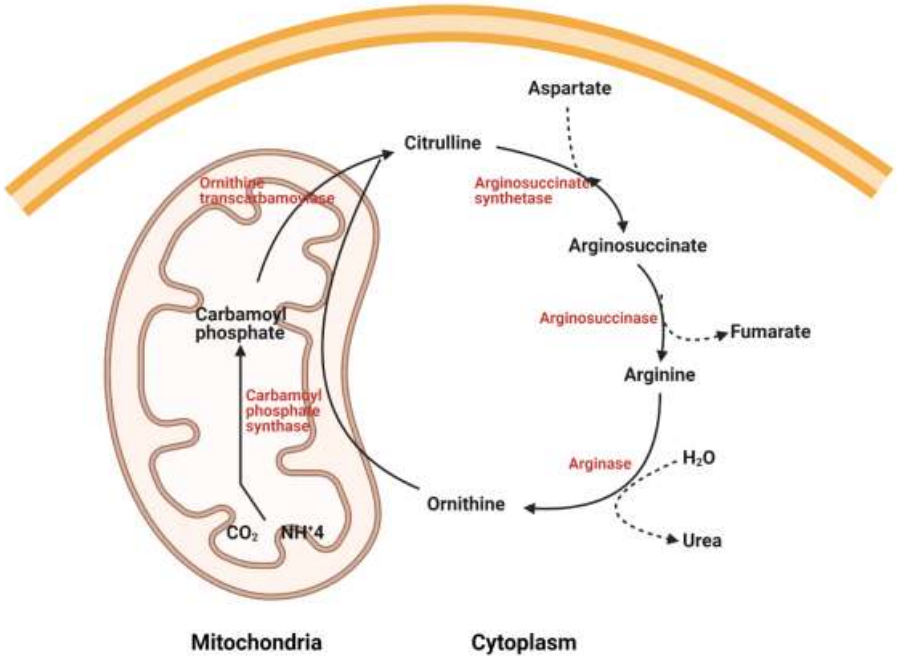



**Fig. (7.14). The urea cycle.** Carbon dioxide and $NH^+4$ are condensed by carbamoyl phosphate synthase, to form the carbamoyl phosphate. Carbamoyl phosphate is condensed with ornithine in the mitochondria *via* ornithine transcarbamoylase to form citrulline. Citrulline enters the cytoplasm and condenses with aspartate *via* arginosuccinate synthetase to form arginosuccinate, which is subsequently split by argininosuccinase into fumarate and arginine. Arginine is then hydrolyzed by arginase by arginase to produce urea and ornithine.

## 7.5. Protein Degradation and Turnover

Proteome integrity is maintained by cellular mechanism networks that monitor folding, concentration, cellular localization, and interactions of proteins from their synthesis through their degradation. The two main mechanisms involved in protein degradation are ubiquitin-proteasome system and autophagy machinery.

### *7.5.1. The Ubiquitin-proteasome System*

The ubiquitin-proteasome (UPS) system is the primary selective proteolytic system in eukaryotes [48]. The degradation of proteins by the UPS is initiated by the sequential conjugation of ubiquitin, a conserved polypeptide of 76 amino acids, to the substrate protein *via* three enzymes [49] (Fig. **7.15**). The ubiquitine activating enzyme E1 activates the carboxyl-terminal glycine residue of the ubiquitin in an ATP-dependent manner. The activated ubiquitin is next transferred to a cysteine site of the ubiquitin-conjugating enzyme E2. Next, the ubiquitin ligase E3 enzyme links the ubiquitin from the E2 enzyme to a lysine residue of the target protein [50]. There are several E2 and E3 enzymes that recognize one or various specific protein motifs [49]. After ubiquitination, the polyubiquitylated protein is recognized (at least four lysine 48-linked ubiquitins are the primary signal for degradation) and degraded by the proteasome.

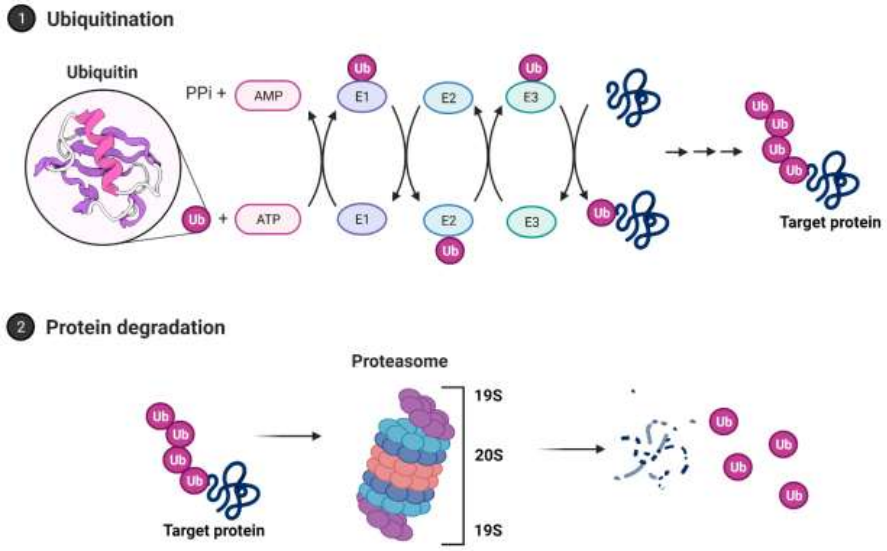



**Fig. (7.15). Ubiquitin-proteasome system**. Protein degradation by UPS starts with the conjugation of ubiquitin to the target substrate protein through E1, E2, and E3. The polyubiquitylated protein is recognized and degraded by the proteasome where the active proteasome is formed by the interaction of the core particle 20S (containing the proteolytic active site) and the regulatory particle.

The proteasome is formed by the assembly of several subunits. The proteasome core particle 20S exhibits a barrel-like structure in which the 28 subunits are assembled into four seven-membered rings. The two outer rings are composed of seven α-subunits (α1- to α7-subunit), and the two inner rings are composed of seven β-subunits (β1- to β7-subunits). b-rings contain the proteolytic active sites: b1, b2 and b5 present caspase-like, trypsin-like and chymotrypsin-like activities, respectively [51]. Oxidized proteins are degraded by proteasome 20S, however, 20S particles are considered to be inactive and unable to degrade other polyubiquitylated proteins [52]. The particle 20S assembles with 19S (26S single capped or 30S double capped) regulatory protein, which recognizes the polyubiquitylated substrate, removes the ubiquitin moieties and unfolds the substrate to translocate it into the 20S proteolytic chamber. The core particle can be activated by other regulatory particles such as PA28 (also known as 11S) complex or the Blm10/PA200 [49].

### *7.5.2. The Autophagy Machinery*

As described in chapter 3, autophagy starts with the formation of the phagophore, a double membrane that can be either newly synthesized or can be originated from the ER, mitochondria or plasma membrane. The phagophore is formed by the complex of ULK, Atg13 and FIP200 [53]. To promote the expansion of the phagophore, PI3K Vps34 complex—Vps15, Vps34, ATG14, Beclin-1, UVRAG, Rubicon—produces phosphatydilinositol-3-phosphate, which is crucial for the formation of the autophagosome [54]. The cytoplasmic fraction is then engulfed into the phagophore. The membrane then elongates until its edges fuse and give rise to the autophagosomes. Conjugation of LC3 to phosphatidylethanolamine, a process known as LC3 lipidation induces phagophore maturation. LC3 lipidation can occur either *via* assembly through the Atg12-Atg5-Atg16L complex or *via* processing of the newly synthesized LC3 through Atg4 to the cytosolic LC3 form (LC3I), and subsequently to the membrane-binding form (LC3II). These reactions are catalyzed through Atg7 and Atg3 in a ubiquitin-like reaction. LC3II-positive autophagosomes are trafficked to the lysosomes through the microtubule network in a dynein-dependent manner [55].

## CONCLUSION

The macronutrients proteins play critical roles in maintaining cellular homeostasis and providing energy. In addition to cellular architectural support, proteins (amino acids) are involved in metabolic reactions, cellular signaling, and several other cellular processes. Their synthesis and degradation are closely regulated by physiological and pathophysiological conditions, including feeding, fasting, physical activities, aging, and disease.

## NOTES

[1] Parietal cells, also known as oxyntic cells are epithelial cells located in the gastric gland in the lining of the fundus and cardia regions of the stomach.

[2] Chief cells, also known as zymogenic or peptic cells are stomach cells releasing pepsinogen and chymosin.

[3] EMS is a rare disorder associated with build up of eosinophils and causes inflammation in different parts of the body including muscle, skin, and lungs.

[4] Transcription is the process by which the information in a strand of DNA is copied into a new molecule of mRNA

## REFERENCES

[1] Rother M, Krzycki JA. Selenocysteine, pyrrolysine, and the unique energy metabolism of methanogenic archaea. Archaea 2010; 2010: 1-14.
[http://dx.doi.org/10.1155/2010/453642] [PMID: 20847933]

[2] Sarasa SB, Mahendran R, Muthusamy G, Thankappan B, Selta DRF, Angayarkanni J. A Brief Review on the Non-protein Amino Acid, Gamma-amino Butyric Acid (GABA): Its Production and Role in Microbes. Curr Microbiol 2020; 77(4): 534-44.
[http://dx.doi.org/10.1007/s00284-019-01839-w] [PMID: 31844936]

[3] Xu S, Gu M, Wu K, Li G. Unraveling the Role of Hydroxyproline in Maintaining the Thermal Stability of the Collagen Triple Helix Structure Using Simulation. J Phys Chem B 2019; 123(36): 7754-63.
[http://dx.doi.org/10.1021/acs.jpcb.9b05006] [PMID: 31418574]

[4] Uzawa K, Yeowell HN, Yamamoto K, Mochida Y, Tanzawa H, Yamauchi M. Lysine hydroxylation of collagen in a fibroblast cell culture system. Biochem Biophys Res Commun 2003; 305(3): 484-7.
[http://dx.doi.org/10.1016/S0006-291X(03)00799-X] [PMID: 12763018]

[5] Gerriets JE, Curwin SL, Last JA. Tendon hypertrophy is associated with increased hydroxylation of nonhelical lysine residues at two specific cross-linking sites in type I collagen. J Biol Chem 1993; 268(34): 25553-60.
[http://dx.doi.org/10.1016/S0021-9258(19)74427-5] [PMID: 8244992]

[6] Harper AE, Benevenga NJ, Wohlhueter RM. Effects of ingestion of disproportionate amounts of amino acids. Physiol Rev 1970; 50(3): 428-558.
[http://dx.doi.org/10.1152/physrev.1970.50.3.428] [PMID: 4912906]

[7] Geliebter AA, Hashim SA, Van Itallie TB. Oral L-histidine fails to reduce taste and smell acuity but induces anorexia and urinary zinc excretion. Am J Clin Nutr 1981; 34(1): 119-20.
[http://dx.doi.org/10.1093/ajcn/34.1.119] [PMID: 6934689]

[8] Hevia P, Kari FW, Ulman EA, Visek WJ. Serum and liver lipids in growing rats fed casein with L-lysine. J Nutr 1980; 110(6): 1224-30.
[http://dx.doi.org/10.1093/jn/110.6.1224] [PMID: 6770059]

[9] Hevia P, Ulman EA, Kari FW, Visek WJ. Serum lipids of rats fed excess L-lysine and different carbohydrates. J Nutr 1980; 110(6): 1231-9.

[http://dx.doi.org/10.1093/jn/110.6.1231] [PMID: 6770060]

[10] Garlick P J. The nature of human hazards associated with excessive intake of amino acids J Nutr 134, 1633S-1639S; discussion 1664S-1666S, 1667S-1672S 2004.
[http://dx.doi.org/10.1093/jn/134.6.1633S]

[11] Steele RD, Barber TA, Lalich J, Benevenga NJ. Effects of dietary 3-methylthiopropionate on metabolism, growth and hematopoiesis in the rat. J Nutr 1979; 109(10): 1739-51.
[http://dx.doi.org/10.1093/jn/109.10.1739] [PMID: 490212]

[12] Benevenga NJ, Smolin LA, Engstrom MA, Steele RD. Methionine toxicity in the chick. J Nutr 1979; 109(9): 1661-3.
[http://dx.doi.org/10.1093/jn/109.9.1661] [PMID: 479959]

[13] Benevenga NJ, Yeh M-H, Lalich JJ. Growth depression and tissue reaction to the consumption of excess dietary methionine and S-methyl-L-cysteine. J Nutr 1976; 106(12): 1715-20.
[http://dx.doi.org/10.1093/jn/106.12.1715] [PMID: 993852]

[14] Wurtman RJ, Hefti F, Melamed E. Precursor control of neurotransmitter synthesis. Pharmacol Rev 1980; 32(4): 315-35.
[PMID: 6115400]

[15] Blauvelt A, Falanga V. Idiopathic and L-tryptophan-associated eosinophilic fasciitis before and after L-tryptophan contamination. Arch Dermatol 1991; 127(8): 1159-66.
[http://dx.doi.org/10.1001/archderm.1991.01680070059006] [PMID: 1863073]

[16] Prensky AL, Fishman MA, Daftari B. Recovery of rat brain from a brief hyperphenylalaninemic insult early in development. Brain Res 1974; 73(1): 51-8.
[http://dx.doi.org/10.1016/0006-8993(74)91006-3] [PMID: 4407391]

[17] Mamunes P, Prince PE, Thornton NH, Hunt PA, Hitchcock ES. Intellectual deficits after transient tyrosinemia in the term neonate. Pediatrics 1976; 57(5): 675-80.
[http://dx.doi.org/10.1542/peds.57.5.675] [PMID: 940707]

[18] Fuda NJ, Ardehali MB, Lis JT. Defining mechanisms that regulate RNA polymerase II transcription in vivo. Nature 2009; 461(7261): 186-92.
[http://dx.doi.org/10.1038/nature08449] [PMID: 19741698]

[19] Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev 2011; 25(10): 1010-22.
[http://dx.doi.org/10.1101/gad.2037511] [PMID: 21576262]

[20] Müller F, Tora L. Chromatin and DNA sequences in defining promoters for transcription initiation. Biochim Biophys Acta Gene Regul Mech 2014; 1839(3): 118-28.
[http://dx.doi.org/10.1016/j.bbagrm.2013.11.003] [PMID: 24275614]

[21] Dynan WS, Tjian R. Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerase II. Cell 1983; 32(3): 669-80.
[http://dx.doi.org/10.1016/0092-8674(83)90053-3] [PMID: 6187469]

[22] Dynan WS, Tjian R. The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. Cell 1983; 35(1): 79-87.
[http://dx.doi.org/10.1016/0092-8674(83)90210-6] [PMID: 6313230]

[23] Engelke D, Ng S, Shastry B, Roeder R. Specific interaction of a purified transcription factor with an internal control region of 5S RNA genes. Cell 1980; 19(3): 717-28.
[http://dx.doi.org/10.1016/S0092-8674(80)80048-1] [PMID: 6153931]

[24] Ptashne M, Gann A. Transcriptional activation by recruitment. Nature 1997; 386(6625): 569-77.
[http://dx.doi.org/10.1038/386569a0] [PMID: 9121580]

[25] Chen HT, Hahn S. Mapping the location of TFIIB within the RNA polymerase II transcription preinitiation complex: a model for the structure of the PIC. Cell 2004; 119(2): 169-80.

[http://dx.doi.org/10.1016/j.cell.2004.09.028] [PMID: 15479635]

[26] Bushnell DA, Westover KD, Davis RE, Kornberg RD. Structural basis of transcription: an RNA polymerase II-TFIIB cocrystal at 4.5 Angstroms. Science 2004; 303(5660): 983-8.
[http://dx.doi.org/10.1126/science.1090838] [PMID: 14963322]

[27] Sainsbury S, Niesser J, Cramer P. Structure and function of the initially transcribing RNA polymerase II–TFIIB complex. Nature 2013; 493(7432): 437-40.
[http://dx.doi.org/10.1038/nature11715] [PMID: 23151482]

[28] Grünberg S, Warfield L, Hahn S. Architecture of the RNA polymerase II preinitiation complex and mechanism of ATP-dependent promoter opening. Nat Struct Mol Biol 2012; 19(8): 788-96.
[http://dx.doi.org/10.1038/nsmb.2334] [PMID: 22751016]

[29] Tsai KL, Yu X, Gopalan S, *et al.* Mediator structure and rearrangements required for holoenzyme formation. Nature 2017; 544(7649): 196-201.
[http://dx.doi.org/10.1038/nature21393] [PMID: 28241144]

[30] Gnatt AL, Cramer P, Fu J, Bushnell DA, Kornberg RD. Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 A resolution. Science 2001; 292(5523): 1876-82.
[http://dx.doi.org/10.1126/science.1059495] [PMID: 11313499]

[31] Wang D, Bushnell DA, Westover KD, Kaplan CD, Kornberg RD. Structural basis of transcription: role of the trigger loop in substrate specificity and catalysis. Cell 2006; 127(5): 941-54.
[http://dx.doi.org/10.1016/j.cell.2006.11.023] [PMID: 17129781]

[32] Bentley DL. Coupling mRNA processing with transcription in time and space. Nat Rev Genet 2014; 15(3): 163-75.
[http://dx.doi.org/10.1038/nrg3662] [PMID: 24514444]

[33] Horman S, Browne GJ, Krause U, *et al.* Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. Curr Biol 2002; 12(16): 1419-23.
[http://dx.doi.org/10.1016/S0960-9822(02)01077-1] [PMID: 12194824]

[34] Pende M, Um SH, Mieulet V, *et al.* S6K1(-/-)/S6K2(-/-) mice exhibit perinatal lethality and rapamycin-sensitive 5′-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. Mol Cell Biol 2004; 24(8): 3112-24.
[http://dx.doi.org/10.1128/MCB.24.8.3112-3124.2004] [PMID: 15060135]

[35] Jefferies HB, Reinhard C, Kozma SC, Thomas G. Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family. Proc Natl Acad Sci USA 1994; 91(10): 4441-5.
[http://dx.doi.org/10.1073/pnas.91.10.4441] [PMID: 8183928]

[36] Jefferies HBJ, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. EMBO J 1997; 16(12): 3693-704.
[http://dx.doi.org/10.1093/emboj/16.12.3693] [PMID: 9218810]

[37] Hannan KM, Brandenburger Y, Jenkins A, *et al.* mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. Mol Cell Biol 2003; 23(23): 8862-77.
[http://dx.doi.org/10.1128/MCB.23.23.8862-8877.2003] [PMID: 14612424]

[38] Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes Dev 2004; 18(4): 423-34.
[http://dx.doi.org/10.1101/gad.285504] [PMID: 15004009]

[39] Frottin F, Martinez A, Peynot P, *et al.* The proteomics of N-terminal methionine cleavage. Mol Cell Proteomics 2006; 5(12): 2336-49.
[http://dx.doi.org/10.1074/mcp.M600225-MCP200] [PMID: 16963780]

[40] Opdenakker G, Rudd PM, Ponting CP, Dwek RA. Concepts and principles of glycobiology. FASEB J 1993; 7(14): 1330-7.

[http://dx.doi.org/10.1096/fasebj.7.14.8224606] [PMID: 8224606]

[41] Ubersax JA, Ferrell JE Jr. Mechanisms of specificity in protein phosphorylation. Nat Rev Mol Cell Biol 2007; 8(7): 530-41.
[http://dx.doi.org/10.1038/nrm2203] [PMID: 17585314]

[42] Cohen P. The regulation of protein function by multisite phosphorylation – a 25 year update. Trends Biochem Sci 2000; 25(12): 596-601.
[http://dx.doi.org/10.1016/S0968-0004(00)01712-6] [PMID: 11116185]

[43] Alonso V, Friedman PA. Minireview: ubiquitination-regulated G protein-coupled receptor signaling and trafficking. Mol Endocrinol 2013; 27(4): 558-72.
[http://dx.doi.org/10.1210/me.2012-1404] [PMID: 23471539]

[44] Zhao J. Sumoylation regulates diverse biological processes. Cell Mol Life Sci 2007; 64(23): 3017-33.
[http://dx.doi.org/10.1007/s00018-007-7137-4] [PMID: 17763827]

[45] Fan GF, Ray K, Zhao X, Goldsmith PK, Spiegel AM. Mutational analysis of the cysteines in the extracellular domain of the human Ca $^{2+}$ receptor: effects on cell surface expression, dimerization and signal transduction. FEBS Lett 1998; 436(3): 353-6.
[http://dx.doi.org/10.1016/S0014-5793(98)01165-X] [PMID: 9801147]

[46] Resh MD. Regulation of cellular signalling by fatty acid acylation and prenylation of signal transduction proteins. Cell Signal 1996; 8(6): 403-12.
[http://dx.doi.org/10.1016/S0898-6568(96)00088-5] [PMID: 8958442]

[47] Karasawa Y. Significant role of the nitrogen recycling system through the ceca occurs in protein-depleted chickens. J Exp Zool 1999; 283(4-5): 418-25.
[http://dx.doi.org/10.1002/(SICI)1097-010X(19990301/01)283:4/5<418::AID-JEZ11>3.0.CO;2-G] [PMID: 10069037]

[48] Schmidt M, Finley D. Regulation of proteasome activity in health and disease. Biochim Biophys Acta Mol Cell Res 2014; 1843(1): 13-25.
[http://dx.doi.org/10.1016/j.bbamcr.2013.08.012] [PMID: 23994620]

[49] Finley D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu Rev Biochem 2009; 78(1): 477-513.
[http://dx.doi.org/10.1146/annurev.biochem.78.081507.101607] [PMID: 19489727]

[50] Ciechanover A. The unravelling of the ubiquitin system. Nat Rev Mol Cell Biol 2015; 16(5): 322-4.
[http://dx.doi.org/10.1038/nrm3982] [PMID: 25907614]

[51] Pines J, Lindon C. Proteolysis: anytime, any place, anywhere? Nat Cell Biol 2005; 7(8): 731-5.
[http://dx.doi.org/10.1038/ncb0805-731] [PMID: 16056263]

[52] Stadtmueller BM, Hill CP. Proteasome Activators. Mol Cell 2011; 41(1): 8-19.
[http://dx.doi.org/10.1016/j.molcel.2010.12.020] [PMID: 21211719]

[53] Egan D, Kim J, Shaw RJ, Guan KL. The autophagy initiating kinase ULK1 is regulated *via* opposing phosphorylation by AMPK and mTOR. Autophagy 2011; 7(6): 643-4.
[http://dx.doi.org/10.4161/auto.7.6.15123] [PMID: 21460621]

[54] Mizushima N, Levine B. Autophagy in mammalian development and differentiation. Nat Cell Biol 2010; 12(9): 823-30.
[http://dx.doi.org/10.1038/ncb0910-823] [PMID: 20811354]

[55] Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell 2011; 147(4): 728-41.
[http://dx.doi.org/10.1016/j.cell.2011.10.026] [PMID: 22078875]

# CHAPTER 8

# Carbohydrate Metabolism

**Abstract:** Carbohydrate metabolism starts with the ingestion of food, breakdown (digestion), and absorption of monosaccharides by the intestinal enterocytes. The absorbed monosaccharides are involved in many cellular processes. They are transferred to cells for aerobic and anaerobic respiration *via* glycolysis, citric acid cycle and pentose phosphate pathway to be used in the starvation state. In the normal state, the skeletal muscle and liver cells store monosaccharides in the form of glycogen. The extra glucose is converted to triglycerides *via* lipogenesis and is stored in the lipid droplets of adipocytes. The present chapter describes in details carbohydrate metabolism and its cellular processes.

**Keywords:** Absorption, Carbohydrates, Digestion, Cellular respiration, Gluconeogenesis, Glycogenolysis, Glycogenesis, Glycolysis, Transport.

## INTRODUCTION

Although the body can generate glucose from amino acids (as described in the previous chapter, gluconeogenesis), there is an average requirement of carbohydrates per day to maintain normal blood glucose levels necessary for the brain- and red blood cell metabolisms, and to avoid the development of ketosis[1]. Also, athletes may derive a large portion of their total energy needs from carbohydrates to optimize performance and recovery. In addition, most dietary fibers are classified as carbohydrates and they play key roles in digestion and health. This chapter will describe carbohydrate types, digestion, absorption, and metabolisms.

### 8.1. Types and Characteristics of Carbohydrates

Carbohydrates were identified as sweet urine in the sixth century BCE by the Indian physician Sushruta [1]. The word “starch” dates from the late fourteen century as stercan, stiercan, or starkjan in Anglo-Saxon Kingdom of Mercia, West Saxon, and German, respectively. The ancient names for sugar, such as sukkar in Arabic, sacchari in Greek, and saccharum in Latin, derived from India where the

**Sami Dridi**

technology of making table sugar was developed and exported. The word carbohydrates (French "hydrate de carbone") literally means carbon with water as the ratio of carbon to water is one to one. In 1838, Jean Baptiste Dumas coined the term glucose (Greek gleukos or sweet wine) for the sugar obtained from honey, starch, and grapes. In 1855, Claude Bernard isolated and coined the term glycogen for starch-like substance stored in the mammalian liver [2]. In 1857, William Miller coined the name sucrose, and in 1866 Friedrich August Kekule proposed the term dextrose (from Latin dexter means right) for dextrorotatory glucose because, in an aqueous solution of glucose, the plane of linearity polarized light is turned to the right. Between 1880-1890, The German chemist Emil Fischer, who received the 1902 Nobel Prize in Chemistry, defined the structure and the chemical make up of glucose.

Although hundreds of different carbohydrates exist in nature, in this chapter, I group them in the most simple way, namely in sugars (monosaccharides and disaccharides), oligosaccharides, and polysaccharides (Fig. **8.1**).

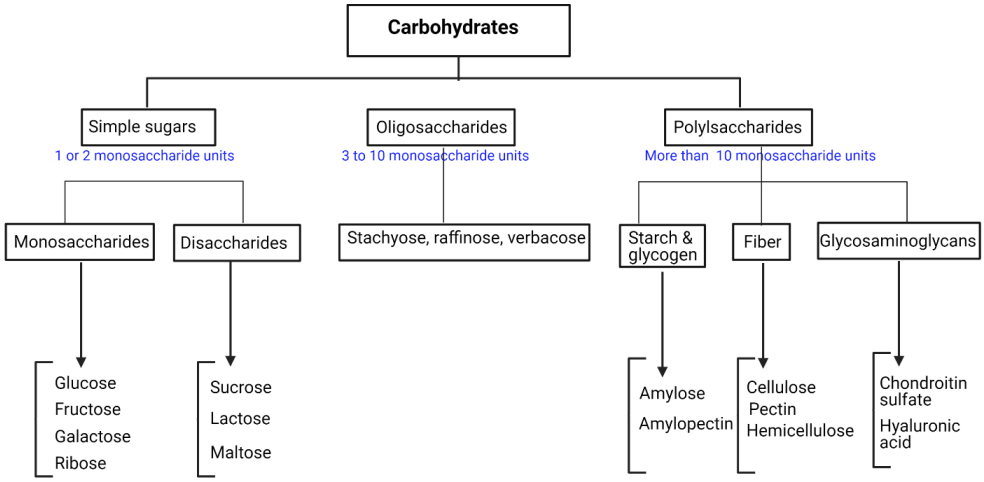



**Fig. (8.1).** Classification of carbohydrates.

## *8.1.1. Monosaccharides*

Based on carbon numbers, trioses, tetroses, pentoses, and hexoses are important in human nutrition. Particularly, glucose, galactose, and fructose are the six-carbon hexoses and the most common monosaccharides used in the human diet. Glucose is the principal carbohydrate found in human circulation (blood). When looking at the monosaccharide as a straight chain, the position of the hydroxyl group on the asymmetric carbon farthest away from the carbonyl group (C=O) is used to

determine the D and L isomer series. If the OH group is on the right side, thus, the monosaccharide is a D-isomer (Fig. **8.2**). If the OH group is on the left side, then the monosaccharide is an L-isomer. The D isomers are the naturally predominant forms and the isomerase racemase enzyme interconverts between the two isomers [3].

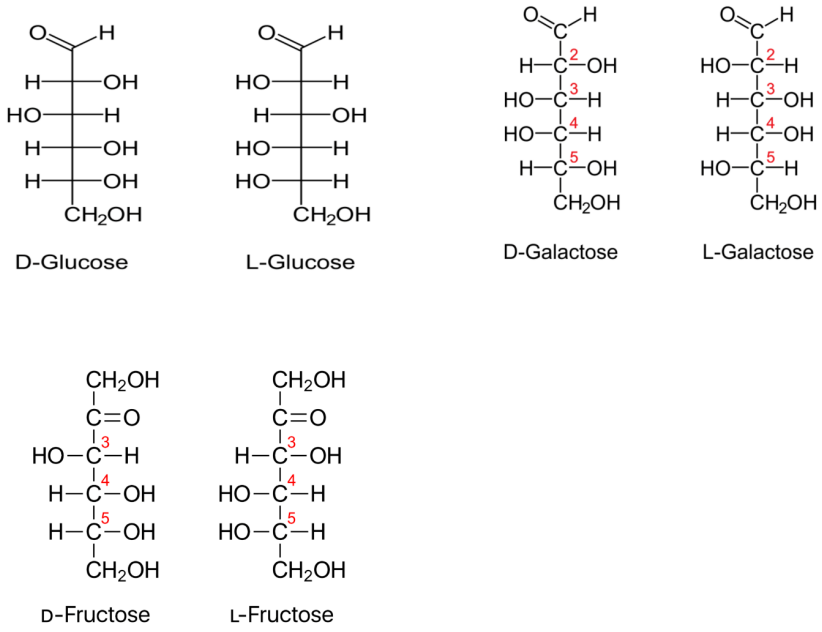



**Fig. (8.2).** Carbohydrate D- and L-isomers.

Based on the groups they contain, monosaccharides can be aldose or ketose. An aldose is defined as a monosaccharide whose carbon skeleton has an aldehyde group, such as glucose, ribose, and galactose. A ketose, however, is a monosaccharide whose carbon skeleton has a ketone group, including fructose, erythrulose, and ribulose.

### *8.1.2. Di- and Polysaccharides*

Disaccharides are composed of two covalently linked monosaccharides. For instance, sucrose (fructose + glucose), lactose (glucose + galactose), and maltose (glucose + glucose), are the three most common disaccharides. Lactose, or milk sugar, is derived from animals however, sucrose and maltose are found in plants.

Oligosaccharides (stachyose, verbascose, raffinose) are composed of 3 to 10 monosaccharide units linked by glycosidic bonds between the OH groups of

adjacent monomeric units. The fate of oligosaccharides is quite different in that they are fermented by colon bacteria. Polysaccharides are composed of a chain of repeating monosaccharide units. In linear chains, the covalent bonds are found between carbons 1 and 4, however, in the branched chains, the bonds are between carbons 1 and 6 (example of glycogen).

## 8.2. Digestion and Absorption of Carbohydrates

Carbohydrate, in the form of refined sugary products, consumption increased steadily during the last few decades. The dietary reference intake (DRI) for carbohydrate energy has been set at 130 grams per day for all people older than one year. This amount is expected to provide 520 calories of energy necessary for the normal function of glucose-dependent tissues such as the brain and red blood cells.

The final goal of carbohydrate digestion is to liberate monosaccharides from disaccharides, oligosaccharides, and polysaccharides and provide the necessary energy for the organism. The digestion of carbohydrates starts with the salivary amylase in the mouth (Fig. **8.3).** Approximately up to 30-40% of the digestion of complex carbohydrates can take place before the food reaches the small intestine. Next, the contents are mixed with the highly acidic gastric juice where the amylase activity is inhibited (optimal pH for amylase is 6.6 - 6.8). In the small intestine, carbohydrate digestion picks up, with most the monosaccharides being absorbed in the small intestine. Pancreatic juice enters the lumen *via* the hepatopancreatic sphincter and neutralizes the gastric acid *via* its high bicarbonate concentration. Pancreatic acinar cells secrete α-amylase that hydrolyses the α1-4 glycosidic linkages of di- and polysaccharides. For instance, dietary starch is hydrolyzed to maltose, maltotriose, α-dextrins, and glucose. In the intestinal lumen, the remainder of carbohydrates are digested by disaccharidases (maltase, lactase, sucrase) which are associated with the microvilli plasma membrane, and the final digested monosaccharides are ready to be absorbed [4]. Table **8.1** summarizes the digestion of some dietary carbohydrates and the brush border membranes enzymes involved in the final digestion step.

The simple monosaccharides (D-isomers but not L-isomers) are transported *via* specific transport proteins. Glucose and galactose are taken up by the $Na^+$-coupled secondary active transport symporter known as $Na^+$-glucose transporter 1 (SGLT1) [5]. Glucose can also reach the circulation *via* a facilitated diffusion transporter, glucose transporter 2 (GLUT2), from a high concentration inside the cell to a low concentration outside the cell [6]. GLUT5 transports fructose. GLUTs are integral membrane transport proteins folded into 12 transmembrane-spanning α-helixes that form a central aqueous channel for the movement of the

monosaccharides across the lipid bilayer [6]. The basolateral $Na^+$, $K^+$-ATPase creates, in coordination with SGLT1, an $Na^+$ gradient to bring hexoses into the enterocytes. As the bloodstream is adjacent to the intestinal epithelial cells, the simple sugar (glucose, galactose, fructose) exit the cells *via* GLUT2 without the use of cellular energy (Fig. **8.4**).

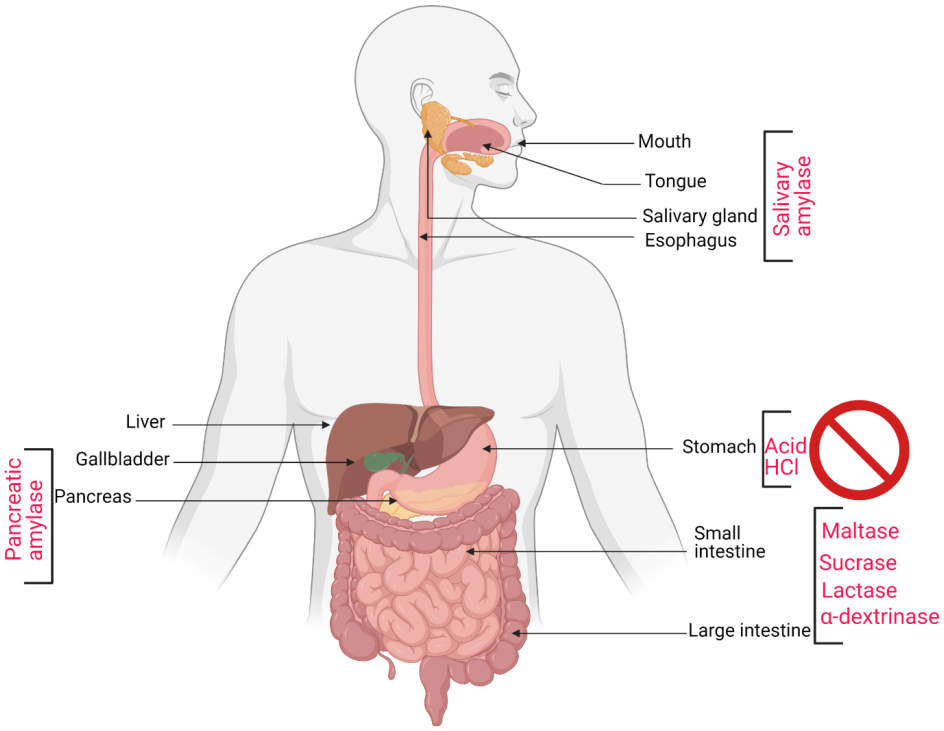



**Fig. (8.3). Carbohydrate digestion.** The digestion begins with the salivary amylase in the mouth and then picks up in the intestine, where the pancreatic amylase hydrolyses the α1-4 glycosidic linkages of di- and polysaccharides. The remainder of carbohydrates is digested in the lumen by disaccharidase enzymes.

**Table 8.1. Carbohydrate source and digestive enzymes.**

| Carbohydrates | Sources | Glycosidic Bonds | Enzymes | Products |
|---|---|---|---|---|
| Fructose | Fruit and honey | None | None | Fructose |
| Glucose | Fruit, honey, grapes | None | None | Glucose |
| Amylopectin | Potatoes, rice, bread | α-1,4 & 1,6 | β-glucoamylase, | Glucose |
| Amylose | Rice, corn, bread | α-1,4 | isomaltase | Glucose |
| Sucrose | Table sugar | α-1,2 | β-glucoamylase | Glucose,fructose |
| Trehalose | Mushrooms | α-1,1 | Sucrase | Glucose |
| Lactose | Milk and milk products | β-1,4 | Trehalase Lactase | Glucose,galactose |

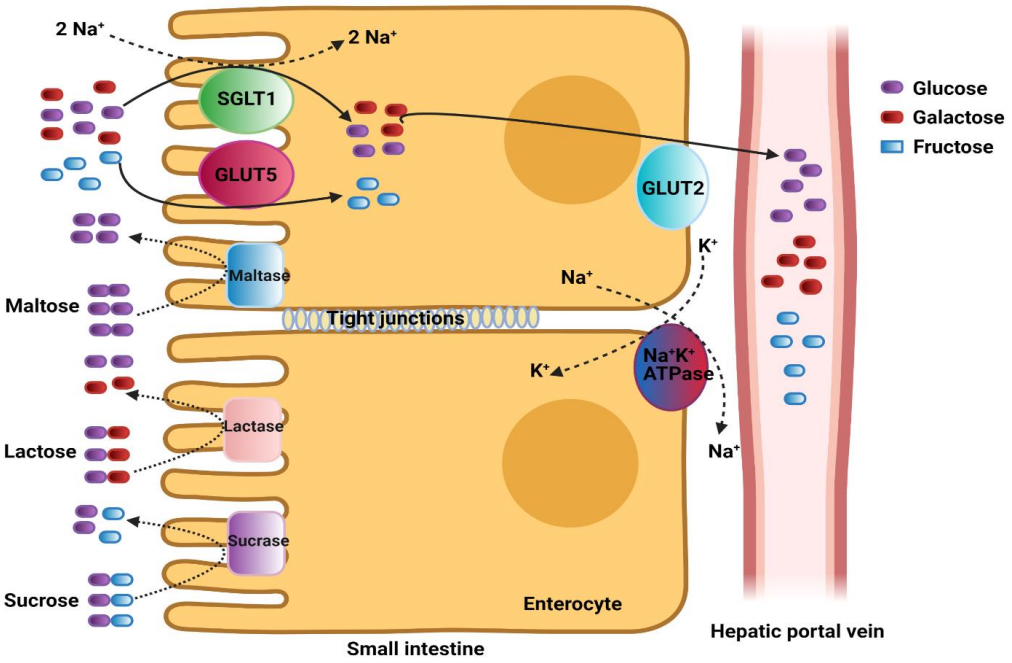



**Fig. (8.4). Absorption of simple sugars.** Disaccharides are digested by brush border disaccharidases (maltase, sucrose, and lactase). Glucose and galactose bind to SGLT1 also binds sodium and enter enterocytes. Fructose enters *via* GLUT5. $Na^+$ and SGLT1 create a Na+ and glucose gradients that coordinates with $Na^+K^+$ ATPase and allow sugars to exit the basolateral side and enter the circulation *via* GLUT2. GLUT, glucose transporter; SGLT, sodium ($Na^+$)-glucose transporter.

## 8.3. Carbohydrate Metabolisms

### *8.3.1. Carbohydrate Uptake*

All cells, particularly the brain, red cells, and muscle, can use glucose for energy purposes. Therefore, circulating glucose needs to reach these cells and cross the plasma membrane (see chapter 2 for cell components). Blood glucose increases after the meal and decreases with fasting. Several hormones regulate blood glucose levels, with the most significant and the most studied being insulin, glucagon, epinephrine, and cortisol. The plasma glucose levels of humans under normal condition is approximately 100 mg/dL, however in overweight individuals, mild hyperglycemia is ~108 mg/dL, intermediate hyperglycemia is ~126 mg/dL and high hyperglycemia is over 155 mg/dL [7]. Incident diabetes is assessed at the earliest clinic visit at which the individual exhibited a blood glucose level of more than 126 mg/dL or reported a diagnosis of diabetes. Chickens (*Gallus gallus domesticus*) are naturally hyperglycemic with an average blood glucose level of approximately 300 mg/dL. Nutritionists use glycemic index

for food, which represents the relative rise in plasma glucose two hours after consuming that food [8]. This is determined in comparison with a standard food (glucose and white bread). For instance, if a given food raises blood glucose level to 50% of the rise caused by the standard (glucose), then the glycemic index of that food is 50. Glycemic index is categorized as low (55 or less), medium (56-69), and high (70 or more). Another index, called a glycemic load, was also used, Glycemic load is determined as follows: GL = glycemic index x digestible carbohydrate in a serving/100. Table **8.2** reports some food glycemic index and load published by Atkinson and co-workers [9].

**Table 8.2. Carbohydrate source and glycemic index and load.**

| Carbohydrate Source | Glycemic Index | Glycemic Load |
|---|---|---|
| Cereal | 42 | 8 |
| Apple juice | 40 | 11 |
| Apples | 38 | 6 |
| Bananas | 52 | 12 |
| Beets | 64 | 5 |
| Cantaloupe | 65 | 4 |
| Carrot | 47 | 3 |
| Couscous | 65 | 23 |
| Grapes | 46 | 8 |
| Green peas | 48 | 3 |
| Macaroni | 47 | 23 |
| Pears | 38 | 4 |
| White rice | 64 | 23 |
| Spaghetti | 42 | 20 |
| Table sugar | 68 | 7 |

During a fed state and after a meal, blood glucose levels rise, enter the pancreatic β-cells (75% are β cells) through GLUT on the plasma membrane, and rapidly stimulate insulin secretion. It has long been considered that there are distinct pools of insulin granules in the β-cells, known as readily releasable pools and reserve pools [10].

An increase in glucose levels triggers rapid insulin exocytosis from the pools by the $K_{ATP}$- and $Ca^{2+}$-dependent mechanisms (Fig. **8.5**). Insulin helps glucose uptake by target cells. Under the fasting state, when the blood glucose drops (low than 80 mg/dL), glucagon is secreted from pancreatic islet α-cells (20% are α-cells). Glucagon circulates unbound in the plasma, binds its receptor on the liver cell membrane, and induces glucose secretion. The secretion of insulin and glucagon is controlled in a reciprocal manner by blood glucose levels. 5% of pancreatic cells are δ-cells that produce somatostatin, which inhibits both insulin and glucagon secretion.

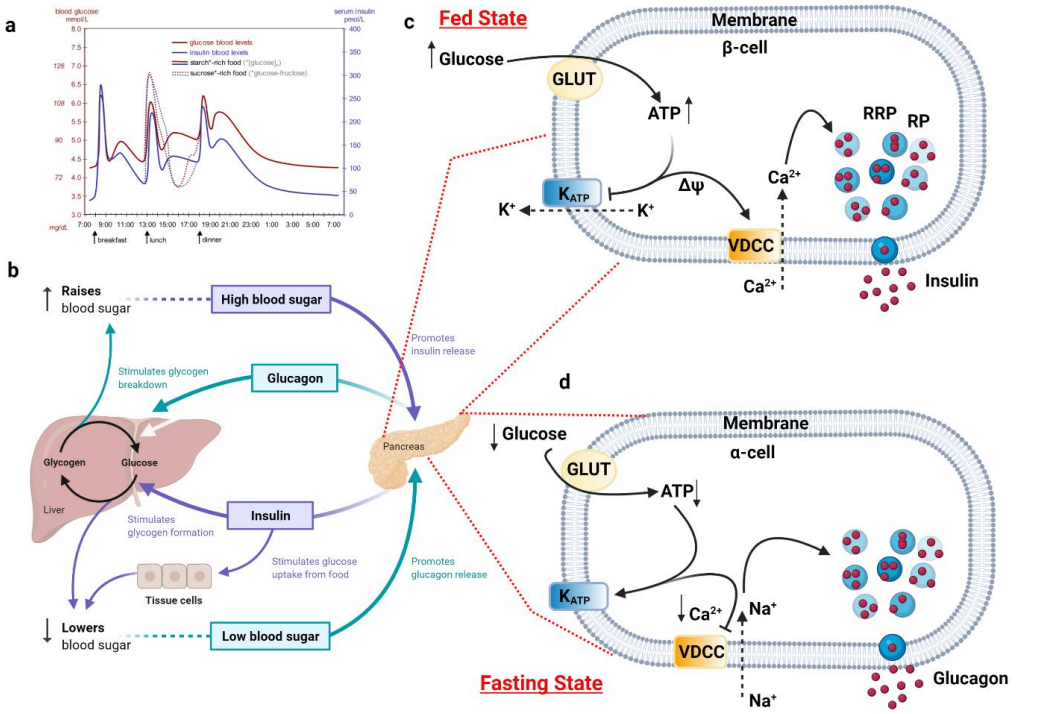



**Fig. (8.5). Effects of glucose concentrations on insulin and glucagon secretion. a)** insulin and glucose secretion after meals, **b)** Interaction between glucose, insulin, and glucagon during fed and fasting states, **c)** and d) mechanisms of glucose action on insulin (fed state) and glucagon (fasting state). ATP, adenosine triphosphate; GLUT, glucose transporter; RP, reserve pools; RRP, readily releasable pools; VDCC, voltage-dependent calcium channel.

## *8.3.1.1. Role and Function of Insulin*

As described above, during the fed state, insulin is secreted and activates anabolic[2] pathways, such as the storage of glycogen and fat. Insulin is secreted in a biphasic manner. The initial burst reflects the release of preformed secretory vesicles; it lasts 5-15 minutes. The second more gradual and sustained secretion (30 min) is due to the release of newly synthesized insulin molecules. Other factors such as an increase in plasma amino acids and the feed forward signaling by glucagon like peptides from the small intestine lead to secretion of insulin. The circulating insulin targets insulin-dependent tissues such as the liver, skeletal muscle, and adipose tissues, regulating glucose uptake and stimulating fuel storage (glycogen and fat).

Insulin promotes the lowering of blood glucose levels by various means. In fat tissue and skeletal muscle, insulin increases the number of GLUTs and induces

their translocation at the plasma membrane. These active GLUTs enhance the uptake of glucose into the muscle and fat cells (Fig. **8.6**). Circulating insulin binds and activates its receptor, which in turn stimulates a complex PI3K–dependent signal transduction network involving insulin receptor substrate (IRS-1) and Akt/protein kinase B and its substrates—the negative regulators AS160 (Akt substrate of 160 kD) and TBC1D1—which regulate Rab8A and Rab14 GTPases involved in muscle cell membrane trafficking [11, 12]. Rab-GTPase–activating proteins AS160 and TBC1D1 inhibit GLUT4 translocation and glucose uptake. Activation of Akt/PKB mediates phosphorylation and inactivation of AS160 and TBC1D1, resulting in the loss of their inhibitory effects on Rab GTPases, thereby allowing GLUT4 translocation and glucose uptake (Fig. **8.6**).

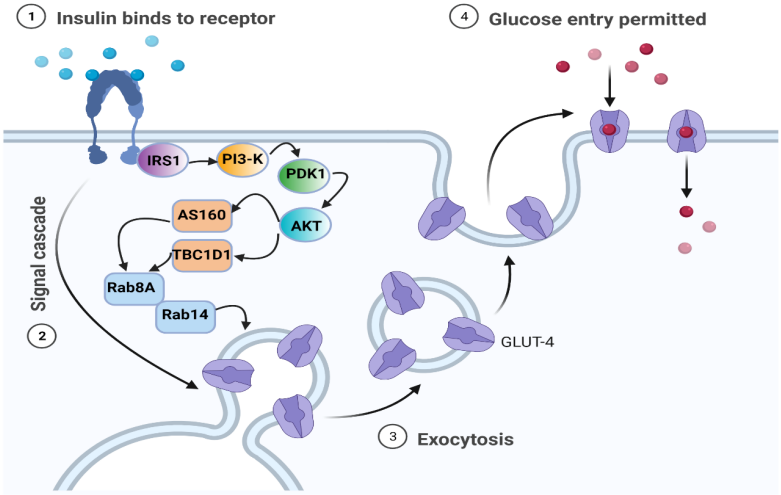



**Fig. (8.6). Role and function of insulin in glucose uptake.** The schematic representation summarizes the major signaling pathways and cascades of insulin involved in insulin-stimulated glucose uptake *via* the activation and translocation of the facilitative GLUT. Akt, protein kinase B; AS160, Akt substrate of 160 kDa; IRS1, insulin receptor substrate 1; PDK1, pyruvate dehydrogenase kinase 1; Rab, ras-related protein; TBC1D1, Tre-2/Bub2/cdc16 1 domain family member 1.

Insulin also facilitates the assembly of Rip11/Rab, exocyst complexes, and soluble NSF attachment protein receptor (SNARE), resulting in docking and fusion of GLUT4-laden vesicles with the plasma membrane [12].

In liver tissue, insulin increases glucose uptake *via* activation and translocation of GLUT2. Once glucose enters hepatocytes, it is quickly phosphorylated by glucokinase, thus maintaining a concentration gradient that further favors the influx of free glucose from the circulation. Table **8.3** summarizes the characteristics of the 14 GLUT family members.

**Table 8.3. Facilitative GLUT transporter family.**

| Gene Name | Protein Name | Substrate | Tissue Distribution |
|---|---|---|---|
| SLC2A1 | GLUT1 | Glucose, galactose, mannose, glucosamine | Erythrocytes, brain, BBB |
| SLC2A2 | GLUT2 | Glucose, galactose, fructose, mannose, glucosamine | Liver, pancreas, intestine, kidney, brain |
| SLC2A3 | GLUT3 | Glucose, galactose, mannose, xylose | Brain,, testes |
| SLC2A4 | GLUT4 | Glucose, glucosamine | Adipose tissue, skeletal and cardiac muscle |
| SLC2A5 | GLUT5 | Fructose | Liver, small intestine, kidney |
| SLC2A6 | GLUT6 | Glucose | Brain, spleen, leucocytes |
| SLC2A7 | GLUT7 | Glucose | Small intestine, colon, testis, prostate |
| SLC2A8 | GLUT8 | Glucose, galactose | Testis, brain, adrenal gland, spleen, adipose tissue, lung |
| SLC2A9 | GLUT9 | Glucose | Kidney, liver, small intestine, placenta, lung |
| SLC2A10 | GLUT10 | Glucose, galactose | Heart, lung, brain, liver, skeletal muscle, pancreas, placenta, kidney |
| SLC2A11 | GLUT11 | Glucose | Heart, muscle |
| SLC2A12 | GLUT12 | Glucose | Heart, prostate, skeletal muscle, placenta |
| SLC2A13 | GLUT13 | *Myo*-inositol | Brain, adipose tissue |
| SLC2A14 | GLUT14 | - | Testis |

### *8.3.1.2. Role and Function of Glucagon*

As described above, an increase in blood glucose levels inhibits glucagon secretion, leading to a fall in plasma glucagon concentrations [13 - 15]. A decrease in plasma glucose levels, however, induces glucagon secretion [16]. Glucagon has profound effects on glucose, amino acid, and fatty acid metabolism that enable survival in conditions such as metabolic stress and starvation. Metabolic actions of glucagon take place predominantly in the liver [17, 18]. Opposed to insulin, glucagon prevents glucose accretion as hepatic glycogen and increases hepatic glucose production during fasting, exercise, and hypoglycemia. Specifically, glucagon promotes the hepatic conversion of glycogen to glucose (glycogenolysis, see section 8.3.2.7), stimulate *de novo* synthesis of glucose (gluconeogenesis, see section 8.3.2.8), and inhibits glucose breakdown (glycolysis, see section 8.3.2.1) and glycogen formation (glycogenesis, see section 8.3.2.6) [19]. Glucagon binds to its receptor, a G protein-coupled receptor, located in the plasma membrane of cells.

The conformation change in the receptor activates G proteins, a heterotrimeric protein with α, β, and γ subunits. When the G protein interacts with the receptor, it undergoes a conformational change that results in the replacement of the GDP molecule that was bound to the α subunit with a GTP molecule. This substitution results in the release of the α subunit from the β and γ subunits (Fig. **8.7**). The alpha subunit specifically activates the adenylate cyclase, the first enzyme and step in the downstream cascade. Adenylate cyclase manufactures cAMP, which in turn activates protein kinase A (PKA), leading to phosphorylation and activation of phosphorylase kinase (PPK) and glycogen phosphorylase to breakdown glycogen to glucose.

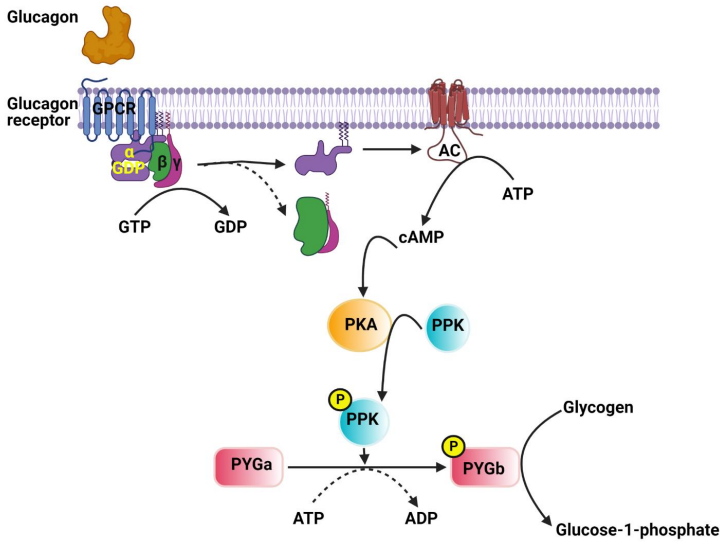



**Fig. (8.7). Role and function of glucagon on hepatic carbohydrate metabolism.** Glucagon binds to its cell membrane receptor and activates G proteins, a heterotrimeric protein with α, β, and γ subunits. When the G protein interacts with the receptor, it undergoes a conformational change that results in the replacement of the GDP molecule that was bound to the α subunit with a GTP molecule. The release of the active α subunit activates AC, which in turn activates PKA *via* cAMP. PKA phosphorylates PPK and activates the glycogen phosphorylase, resulting glycogen breakdown. AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GPCR, G protein coupled receptor; PKA, protein kinase A; PPK, phosphorylase kinase; PYG, glycogen phosphorylase.

### *8.3.1.3. Role and Function of Epinephrine*

Epinephrine (adrenaline), released from nerve endings and the adrenal gland, stimulates glucose production during exercise [20]. As for glucagon, epinephrine binds to its receptor on the cell membrane and activates cAMP-PKA pathway and its downstream cascades, leading to phosphorylation and activation of glycogen phosphorylase and breakdown of glycogen to glucose to be used in the glycolysis process and provide the necessary energy.

### *8.3.2. Fate of Intracellular Glucose and Major Metabolic Pathways*

The final destination of carbohydrates is to provide the necessary energy for the cell. Although most cells use more than one fuel source, red blood cells are the only true obligate glucose users. Under normal conditions, the brain derives nearly all of its necessary energy from glucose. However, the brain can adapt to use more ketone bodies during starvation to spare blood glucose for other tissues.

#### ***8.3.2.1. Glycolysis***

Glycolysis is a series of ten reactions that convert one six-carbon glucose molecule to two three-carbon pyruvate molecules, ATP, NADH, and water. The process takes place in the cytosol of the cell. The pathway of glycolysis and its associated enzymes are shown in Fig. (**8.8**). The summarized key steps in the pathway are:

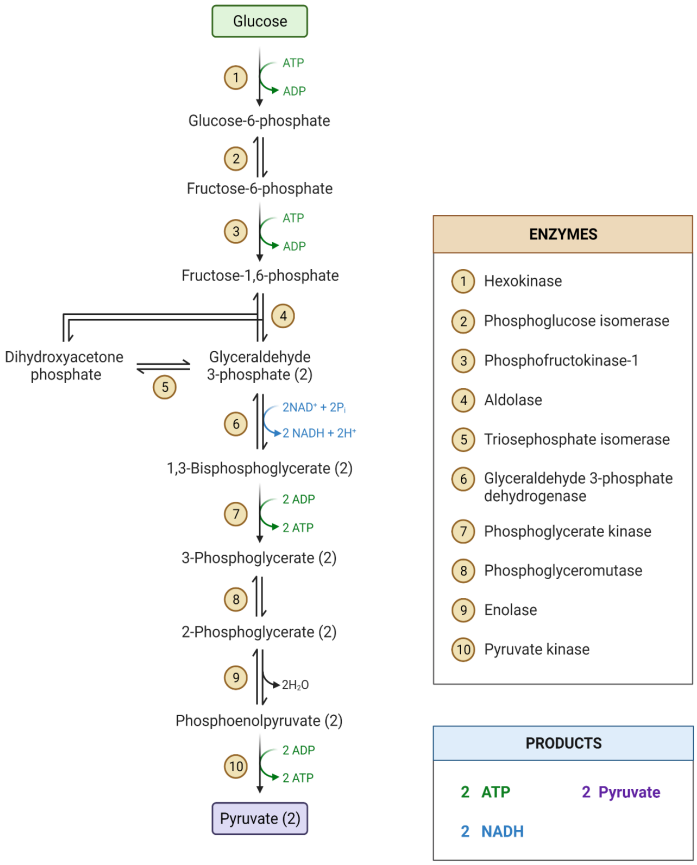



**Fig. (8.8).** Glycolysis and glycolytic enzymes.

Step 1: A phosphate group is added to glucose in the cell cytoplasm by the action of enzyme hexokinase (in all tissues) and glucokinase (in hepatocytes). In this step, a phosphate group is transferred from ATP to glucose, forming glucose---phosphate.

Step 2: The enzyme phosphoglucomutase isomerizes glucose-6-phosphate into fructose-6-phosphate.

Step 3: The enzyme phosphofructokinase transfers a phosphate group from ATP to fructose-6-phosphate and converts it into fructose 1,6-bisphosphate.

Step 4: The enzyme aldolase converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which are isomers of each other.

Step 5: The enzyme triose-phosphate isomerase converts dihydroxyacetone phosphate into glyceraldehyde 3-phosphate, which is the substrate in the successive step of glycolysis.

Step 6: This step contains two reactions:

- The enzyme glyceraldehyde 3-phosphate dehydrogenase transfers one hydrogen molecule from glyceraldehyde phosphate to nicotinamide adenine dinucleotide to form NADH + $H^+$.

- The enzyme glyceraldehyde 3-phosphate dehydrogenase adds a phosphate to the oxidized glyceraldehyde phosphate to form 1,3-bisphosphoglycerate.

Step 7: The enzyme phosphoglycerokinase transfers a phosphate from 1,3-bisphosphoglycerate to ADP to form ATP and two molecules of phosphoglycerate.

Step 8: The phosphate of both the phosphoglycerate molecules is relocated from the third to the second carbon to yield two molecules of 2-phosphoglycerate by the action of phosphoglyceromutase enzyme.

Step 9: The enzyme enolase removes a water molecule from 2-phosphoglycerate to form phosphoenolpyruvate.

Step 10: A phosphate from phosphoenolpyruvate is transferred to ADP to form pyruvate and ATP by the action of pyruvate kinase. Two molecules of pyruvate and ATP are obtained as the end products.

The final reaction (reactants and end products):

$$\text{Glucose} + \text{NAD}^+ + 2\text{ATP} \longrightarrow 2 \text{ pyruvate} + 2 \text{ NADH} + 4 \text{ ATP}$$

. The pyruvate will enter the Krebs cycle and the NADHs will go to the mitochondrial electron transport chain (ETC).

## *8.3.2.2. Fate of Pyruvate*

Depending on the nutritional state, metabolic condition, and cell type, pyruvate can enter the mitochondria and be converted to Acetyl-CoA or oxaloacetate or converted to alanine or lactate in the cytosol. The conversion of pyruvate to acetyl-CoA undergoes a three-step process: 1) A carboxyl group is removed from pyruvate, releasing a molecule of carbon dioxide into the surrounding medium, and two-carbon hydroxyethyl group are formed and bound to the pyruvate dehydrogenase enzyme. 2) The hydroxyethyl group is oxidized to an acetyl group, forming an NADH from $NAD^+$. The NADH will be used by the ETC. 3) The enzyme-bound acetyl group is transferred to CoA, producing a molecule of acetyl-CoA, which will be used in the Krebs cycle (Fig. **8.9**).

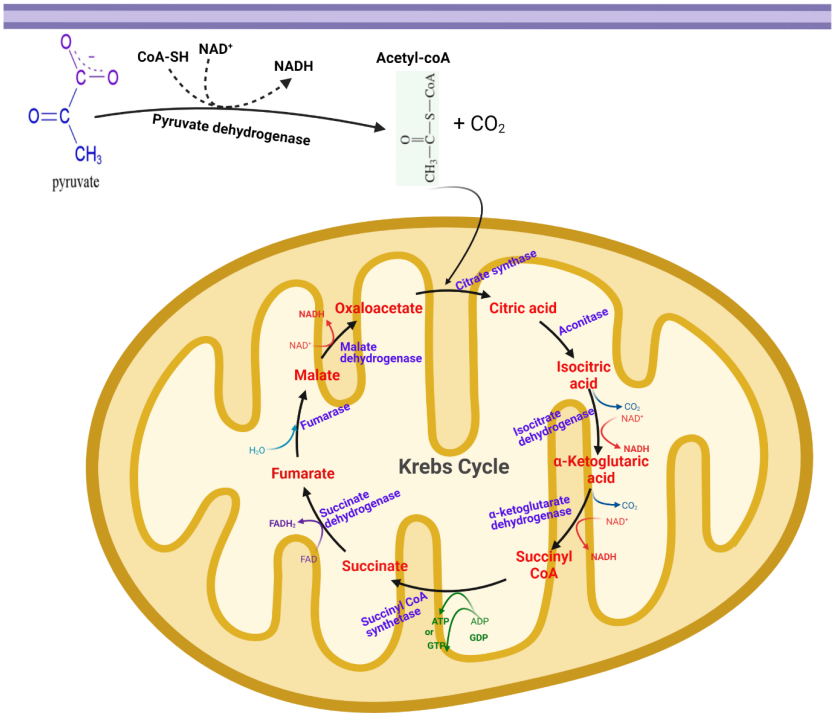



**Fig. (8.9). Pyruvate metabolism and Krebs cycle.** The Krebs cycle is also known as citric acid cycle or tricarboxylic acid cycle.

The Krebs cycle (discovered by Hans Adolf Krebs, the winner of the Nobel Prize of physiology or Medicine in 1953), also known as a citric acid cycle or tricarboxylic acid cycle (TCA), occurs in the mitochondrial matrix where the acetate of acetyl-CoA undergoes stepwise oxidation to carbon dioxide and water in a cyclic pathway. Eight key sequential reactions occur, whereby the final reaction produces the reactant for the first reaction that forms citric acid (Fig. **8.9**). The citrate synthase enzyme catalyzes the condensation of oxaloacetate and acetyl-CoA to form citric acid. The aconitase enzyme catalyzes the conversion of citric acid to isocitrate, which is converted to α-ketoglutarate by the isocitrate dehydrogenase enzyme. The enzyme α-ketoglutarate dehydrogenase converts α-ketoglutarate to succinyl-CoA. During these two oxidative reactions, $NAD^+$ is reduced to NADH and two $CO_2$ molecules are produced (Fig. **8.9).** Next, succinyl-CoA is converted to succinate *via* the action of succinyl-coA synthetase, where energy (ATP) is liberated by the cleavage of the high-energy thioester bond of succinyl-CoA. Succinate is then converted to fumarate by succinate dehydrogenase and here, FAD is reduced to $FADH_2$. The enzyme fumarase catalyzes the conversion of fumarate to malate, which is then converted to oxaloacetate by malate dehydrogenase, and here $NAD^+$ is reduced to NADH. During the TCA cycle, there are four oxidizing reactions, with three producing NADH from $NAD^+$ and one producing $FADH_2$ from FAD. A grand total of 2 ATP are produced by glycolysis and 2 ATP by Krebs cycle. The rest of ATP are produced in the ETC (see section 8.3.2.4).

### ***8.3.2.3. Transfer of Cytosolic NADH into the Mitochondria***

The inner membrane of the mitochondria is impermeable to NAD, and thus the cytosolic NADH produced from glycolysis cannot enter the mitochondria for re-oxidation and needs shuttles. 1) The malate-aspartate shuttle, which involves the reduction of oxaloacetate to malate in the cytosol. Malate enters the mitochondria and is reduced back to oxaloacetate to reduce $NAD^+$ to NADH. Because it cannot cross the mitochondrial inner membrane, oxaloacetate undergoes a transamination by aspartate transaminase to produce aspartate in the presence of glutamate acting as an amino donor, which leads to the production of α-ketoglutarate. Mitochondrial aspartate leaves the mitochondria to the cytosol in exchange for entering glutamate. In the cytosol, aspartate is reverse transaminated by aspartate transaminase to produce oxaloacetate and then malate (Fig. **8.10**). 2) The glycerophosphate shuttle, which involves the reduction of dihydroxyacetone phosphate to glycerol 3-phosphate and oxidation of NADH to $NAD^+$. The glycerol 3-phosphate enters the mitochondria, where it is converted to dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase and reduction of FAD to $FADH_2$ (Fig. **8.10**). Dihydroxyacetone phosphate and glycerol 3-phosphate are

transported in the mitochondria in the opposite direction by an antiporter. The glycerophosphate shuttle is important in the muscle where there is a high glycolysis rate; however, the malate-aspartate shuttle is important in the liver. The malate-aspartate shuttle is sensitive to the $NADH/NAD^+$ ratio in both compartments (cytosol and mitochondria), and cannot function if this ratio in the mitochondria is higher than that in the cytosol. The glycerophosphate shuttle uses the $FADH_2/FAD$ and thus, it can operate independently of the $NADH/NAD^+$ ratio.

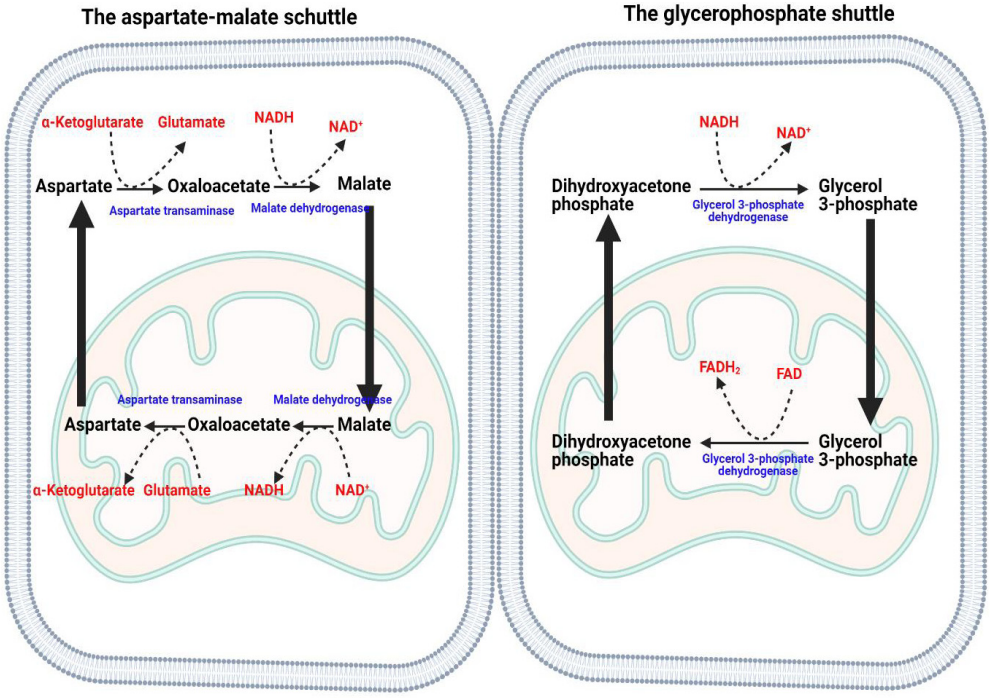



**Fig. (8.10). The aspartate-malate and glycerophosphate shuttles.** FAD, Flavin adenine dinucleotide; $FADH_2$, dihydroflavine-adenine dinucleotide; $NAD^+$, nicotinamide adenine dinucleotide; NADH, Nicotinamide adenine dinucleotide hydrate.

## *8.3.2.4. Mitochondrial Electron Transport Chain*

The ETC, including the transmembrane protein complexes I-IV and the freely mobile electron transporters ubiquinone and cytochrome, is a series of enzymes and coenzymes in the crista membrane [21, 22]. There are two ETC pathways for electron transport: complex I/III/IV using NADH as substrate and complex II/III/IV using succinic acid as substrate.

The respiratory complex I, also known as NADH-ubiquinone oxidoreductase, transfers electrons from matrix NADH to ubiquinone [23]. This complex is composed of 14 core subunits and 45 identified proteins that participate in the

formation of the core subunits [24]. The N2 cluster is the final electron accepting iron-sulfur cluster that delivers electrons to the ubiquinone binding site, which is located at the junction of the membrane arm and matrix arm and in which ubiquinone (C0Q) is reduced to ubiquinol ($QH_2$). This transfer of electrons (2) from NADH to CoQ in complex I induce the pumping of four protons from the matrix into the intermembrane space [25, 26]. The complex II, also known as the succinate dehydrogenase, which is also a component of the Krebs cycle, serves as an association between metabolism and cellular respiration of oxidative phosphorylation (OXPHOS) [27]. The complex II consists of 4 subunits, two containing the CoQ binding site and two containing the succinate binding site. *Via* FeS clusters, complex II donates the electrons from succinate to CoQ. After receiving electrons, FAD is reduced to $FADH_2$. The complex III, also known as cytochrome bc1 complex or CoQ-cytochrome c reductase, transfers the electrons carried by QH2 to cytochrome c. It is a dimer containing 11 subunits per monomer, and the electron transfer process is accomplished by the Q-cycle [28] and two protons are released into the mitochondrial intermembrane space from the matrix. The complex IV, also known as the cytochrome c oxidase, transfers electrons from cytochrome c to terminal electron acceptor $O_2$ to produce $H_2O$. It consists of 13 subunits containing four redox-active metal centers, $Cu_A$, heme a, heme a3, and $Cu_B$ [29]. This leads to the reduction of $O_2$ to $H_2O$, transfer of 4 electrons, and 8 protons are removed from the matrix, of which half is used to form the 2 water molecules, and the other half is pumped into the intermembrane space (Fig. **8.11**). The final complex, complex V also known as the F1/F0 ATP synthase, which consists of two functional domains, F0 and F1. F0 is located in the inner mitochondrial membrane and contains several subunits. However, F1 is located in the mitochondrial matrix and contains several soluble subunits. To generate one $H_2O$ molecule, the ETC transfers 2 electrons at a time to oxygen, which is accompanied by the pumping from the matrix into the intermembrane space of 4 $H^+$ (*via* complex I), 4 $H^+$ (complex III), and 2 $H^+$ (complex IV) or 0, 4, and 2 $H^+$*via* complex II, complex III, and complex IV. During their passage *via* F0, the protons create an electrochemical gradient causing conformational changes of F1/F0 ATP synthase, resulting in ADP phosphorylation and ATP production. In total, 34 ATP are formed by ETC from one molecule of oxygen. In conclusion, one molecule of oxygen produces (36-38 ATP) as follows: 2 ATP from glycolysis, 2 ATP *via* Krebs cycle, and 34 ATP *via* ETC. It is worth noting that the transport of 2 NADH produced by glycolysis from the cytosol to the mitochondria requires 2 ATP, thus the true grant total is 36 ATP per one glucose molecule.

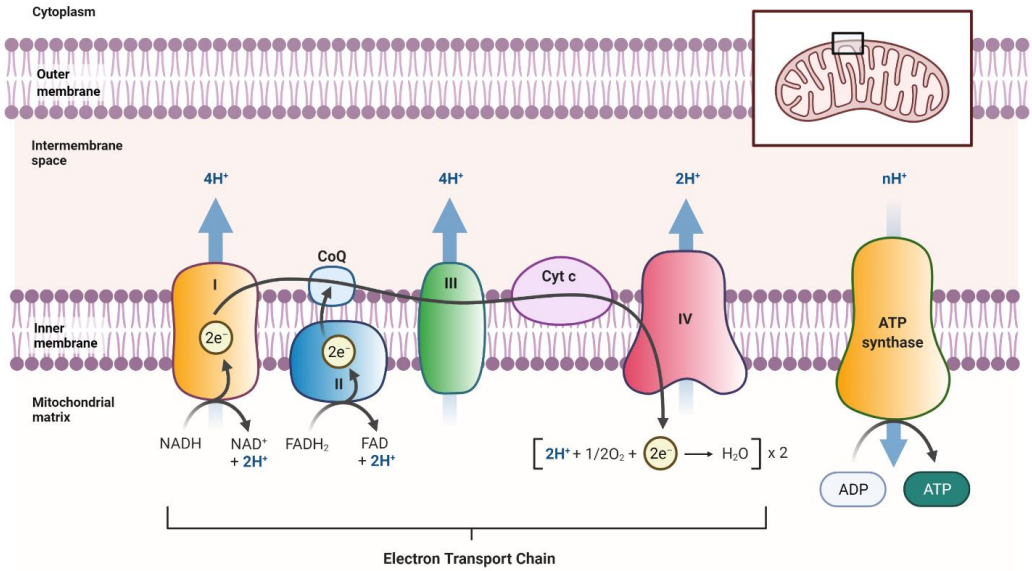



**Fig. (8.11). Schematic representation of mitochondrial ETC.** Electrons derived from oxidizable substrates are passed through complex I/III/IV or complex II/III/IV in an exergonic process that drives the proton pumping into the mitochondrial intermembrane space. The energy of the proton gradient drives the synthesis of ATP from ADP *via* F1F0 ATP synthase.

### *8.3.2.5. Anaerobic Glycolysis*

Under the condition of intense exercises, such as sprinting, the rate of oxygen transport into the muscle is not enough to allow the re-oxidation of all the NADH generated from the glycolysis process. To maintain the oxidation of glucose, pyruvate is converted to lactate, and NADH is oxidized to $NAD^+$. This is also critical for erythrocytes, which lack mitochondria, to re-oxidize NADH. Muscle and erythrocyte lactate is transported to the liver, where it is converted to pyruvate by lactate dehydrogenase (Cori cycle or lactic acid cycle discovered by Carl Ferdinand and Gerty Cori, who won the 1947 Nobel prize in Physiology or Medicine) (Fig. **8.12**), and the pyruvate is used in the gluconeogenesis process to produce glucose.

### *8.3.2.6. Glycogenesis*

During feed restriction or during the submaximal exercise of high intensity, glycogen is the preferred and primary source of energy. Glycogen is a highly ramified polymer of glucose found and stored primarily in the cells of the liver and skeletal muscle. In the liver, glycogen represents 5-6% of the organ's fresh weight (100-200 g for a 1.5 kg liver). In muscle, glycogen can make up 1-2% of

the muscle mass (~400g for 70 kg-adult). Small amounts of glycogen are also found in the kidney, red blood cells [30], white blood cells [31], glia and brain [32]. The uterus also stores glycogen during pregnancy to nourish the embryo [33]. Originally, Claude Bernard communicated to the Société de Biology in Paris, on March 21, 1857, a description of glycogen (sugar-forming substance) isolation from liver tissues. Later on, Sanson A. found that muscle tissues also contain glycogen. In 1858, Kekuke established the empirical formula of glycogen.

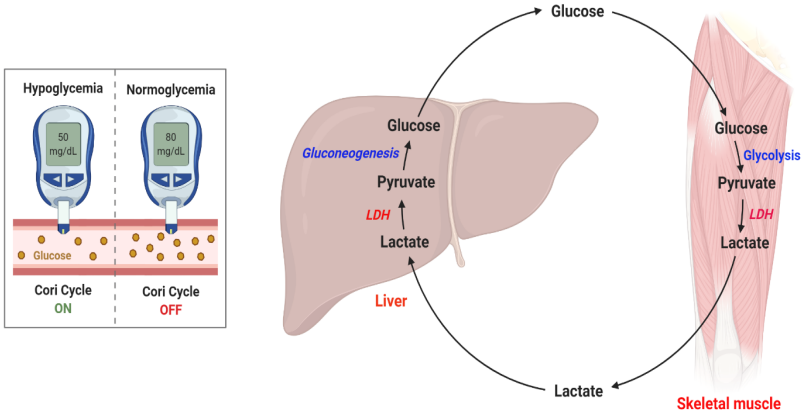



**Fig. (8.12). The Cori cycle or glucose-lactate cycle.** Glucose is converted to lactate in muscle by anaerobic glycolysis. Circulating lactate reaches the liver and is converted to pyruvate by lactate dehydrogenase, and pyruvate enters the gluconeogenesis to produce glucose. LDH, lactate dehydrogenase.

Glycogen is a highly ramified and branched polymer consisting of linear chains of glucose residues with further chains branching off every 8 to 12 glucoses or so. Glucoses are linked together linearly by α(1→4) glycosidic bonds from one glucose to the next (Fig. **8.13**). Branches are linked to the chains from which they are branching off by α(1→6) glycosidic bonds between the first glucose of the new branch and glucose on the stem chain [34] (Fig. **8.13**). Glycogen synthesis or glycogenesis is a multi-step process that begins with the conversion of glucose to glucose-6-phosphate ($G_6P$) *via* muscle hexokinase or hepatic glucokinase. $G_6P$ subsequently converts to glucose-1-phosphate ($G_1P$) *via* phosphoglucomutase. $G_1P$ is converted to UDP glucose *via* glucose-1-phosphate uridyltransferase, which requires UTP as an additional substrate and release of pyrophosphate (PPi) [35]. Glycogenin synthesizes the initial primer for glycogen synthase by attaching a UDP-glucose molecule at its one carbon position to a hydroxyl group on a tyrosine residue [36] (Fig. **8.13**), which causes UDP to exit. Glycogen synthase creates an α-1,4 glycosidic bond between UDP-glucose and the growing glycogen strand [37]. After the linear chain grows (8-12 glucose molecules), the glycogen branching enzyme, which contains two catalytic activities (transferase and α-1,6 glycosidase) begins to add branches [38].

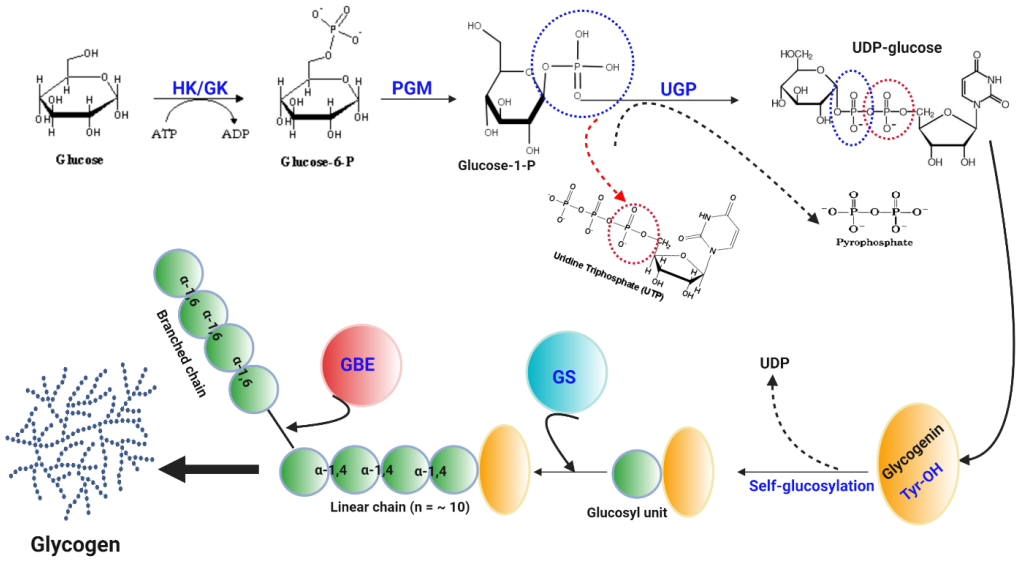



**Fig. (8.13). Schematic representation of glycogenesis.** For glycogen synthesis to proceed, the first few glucose residues are attached to a protein known as glycogenin. Glycogenin catalyzes its own glycosylation, attaching carbon 1 of a UDP-glucose to a tyrosine residue (Y194) on the enzyme. This reaction is carried out by one subunit adding the glucose to the other subunit. Following the addition of the first glucose residue, each glycogenin subunit will then add a further 6-17 glucose residue in an intra-subunit reaction *via* α-1,4 glycosidic bonds. The attached glucose then serves as the primer required by glycogen synthase to attach additional glucose molecules. The glycogen branching enzyme, also known as the amylo-(1,4 to 1,6)-transglucosidase transfers a terminal fragment of 6-7 glucose residues (from a polymer at least 11 glucose residues long) to an internal glucose residue at the C-6 hydroxyl position. GK, glucokinase; GBE, glycogen branched enzyme; GS, glycogen synthase; HK, hexokinase; PGM, phosphoglucomutase; UGP, glucose--phosphate uridyltransferase.

Glycogen is synthesized during the fed state, and it is regulated by a myriad of hormones and factors, with the most known and studied being insulin and glucagon, which promote anabolism and catabolism[3], respectively [39]. As described previously, insulin activates protein phosphatase 1 (PP1) and PKB. PP1 dephosphorylates glycogen synthase b and converts it to the active glycogen synthase a. PKB maintains the active glycogen synthase a by inactivating glycogen synthase kinase 3 (GSK3) [40]. In the glucagon-mediated pathway, PKA phosphorylates PP1 and induces glycogen breakdown by preventing PP1 from activating glycogen synthase b to glycogen synthase a. PKA also activates glycogen phosphorylase kinase and glycogen phosphorylase, which catalyzes the sequential phosphorolysis of glycogen to release $G_1P$ [41]. More recently, leptin has been shown to mimic the effect of insulin on glucose transport and glycogen synthesis through PI3K activity [42, 43], janus kinase (JAK) and insulin receptor substrate 2 (IRS2) [44].

## *8.3.2.7. Glycogenolysis*

Glycogen degradation or breakdown (glycogenolysis) is catalyzed by the active enzyme glycogen phosphorylase a and the debranching enzyme (Fig. **8.14**). Glycogen is cleaved from the non-reducing ends of the chain by the enzyme glycogen phosphorylase, in the presence of phosphate (Pi) and pyridoxal phosphate (a cofactor derived from vitamin B6), to produce monomers of glucose-1-phosphate, which is then converted to glucose-6-phosphate by phosphoglucomutase [45] (Fig. **8.14**). As the glycogen phosphorylase is unable to cleave α-1,4 bonds close to the junction point, a special debranching enzyme, which contains two catalytic properties: a transferase and an α-1,6 glucosidase, is needed to transfer the three distal glucose molecules to a proximal longer chain and hydrolysis of the α-1,6 glycosidic bond, respectively. The hydrolysis of the α-1,6 glycosidic bond yields a glucose unit instead of $G_1P$. The ratio of $G_1P$ to glucose generated from glycogenolysis is 10:1. $G_1P$ produced from glycogenolysis is converted to $G_6P$ by phosphoglucomutase. The $G_6P$ monomers produced have three possible fates: 1) $G_6P$ can continue on the glycolysis pathway and be used as fuel. 2) $G_6P$ can enter the pentose phosphate pathway *via* the enzyme glucose-6-phosphate dehydrogenase to produce NADPH and 5-carbon sugars. In the liver and kidney, $G_6P$ can be dephosphorylated back to glucose by the enzyme glucose 6-phosphatase. This is the final step in the gluconeogenesis pathway. It is important to mention that skeletal muscle does not express glucose-6-phosphatase, and thus uses its glycogen reserve for itself and not as glucose for other tissues [46].

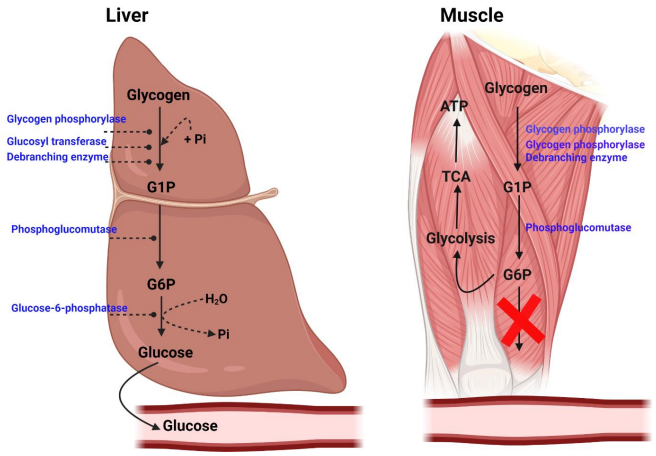



**Fig. (8.14). Schematic representation of glycogenolysis in muscle and liver.** The breakdown of hepatic glycogen produces glucose which circulates and is used by other tissues. The degradation of muscle glycogen, however, is used in glycolysis and energy production for the muscle.$G_1P$, glucose 1-phosphate; $G_6P$, glucose 6-phosphate; TCA, tricarboxylic acid cycle.

### *8.3.2.8. Gluconeogenesis*

Gluconeogenesis, *de novo* synthesis of glucose, is a multistep metabolic process to generate glucose from non-carbohydrate source, mainly from pyruvate or related three-carbon compound lactate and glutamine. It is effectively the glycolysis in reverse (Fig. **8.15**). It has four irreversible steps catalyzed by the enzymes: pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase (FBP), and glucose 6-phosphatase. The role of the gluconeogenesis process is to maintain blood glucose when glycogen stores are depleted and supply the brain and red blood cells that primarily use glucose for energy. It occurs primarily in liver, and to less extent in kidney, enterocytes, and brain, and not in the muscle, which lacks $G_6P$. The liver does not use gluconeogenesis for its energy but uses β-oxidation to supply the necessary energy for glucose *de novo* synthesis. Amino acids, except leucine and lysine, lactate (produced in anaerobic glycolysis), glycerol-3-phosphate (produced in fat catabolism), and propionyl-CoA (produced in odd-carbone fatty acid catabolism) are all substrates for gluconeogenesis.

### *8.3.2.9. The Pentose Phosphate Pathway*

The pentose phosphate pathway (PPP), also known as the hexose monophosphate shunt or pentose phosphate shunt, is an alternative pathway for the conversion of glucose-6-phosphate to fructose-6-phosphate and glyceraldehyde 3-phosphate [47, 48]. Unlike glycolysis and glucose aerobic oxidation, the PPP does not provide ATP as energy, but it supplies NADPH and ribose 5-phosphate. NADPH is the reducing power required for the synthesis of sterols, fatty acids, nucleotides, and non-essential amino acids [49]. NADPH is also involved in glutathione metabolism and cellular antioxidant defense, as well as reactive oxygen species metabolism [50]. The ribose 5-phosphate is the building block for nucleic acid synthesis.

The PPP is subdivided, by a series of enzymes, into two biochemical branches, known as the oxidative and non-oxidative branches, all of which occur in the cytosol (Fig. **8.16**). Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme of the oxidative PPP, determining the flux of $G_6P$ directed into the pathway.

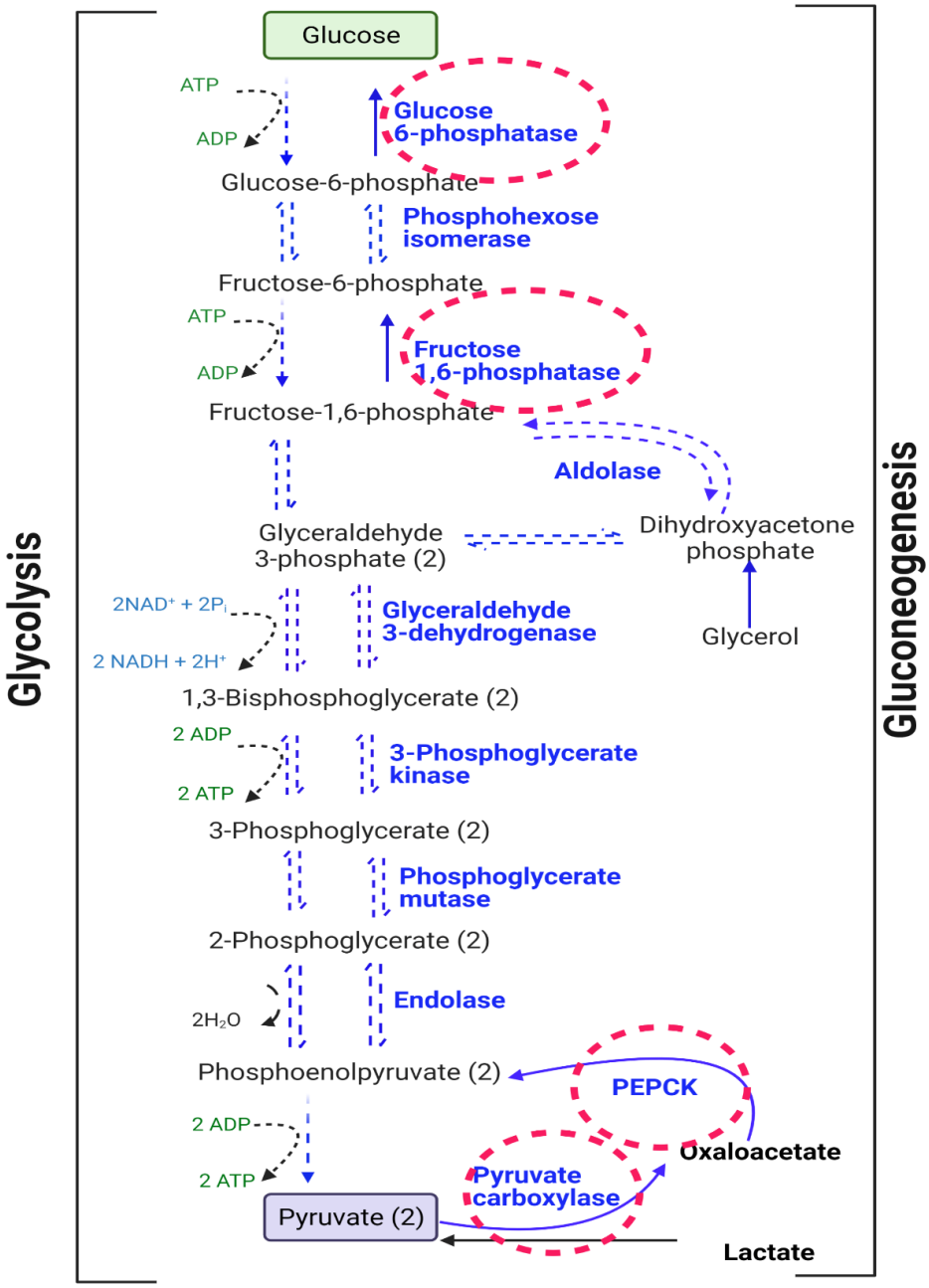



**Fig. (8.15). Schematic representation of gluconeogenesis.** Gluconeogenesis is a *de novo* synthesis of glucose from non-carbohydrate source. It is effectively the glycolysis in reverse, with four irreversible steps catalyzed by the enzymes: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase (circled by the red line). PEPCK, phosphoenolpyruvate carboxykinase.

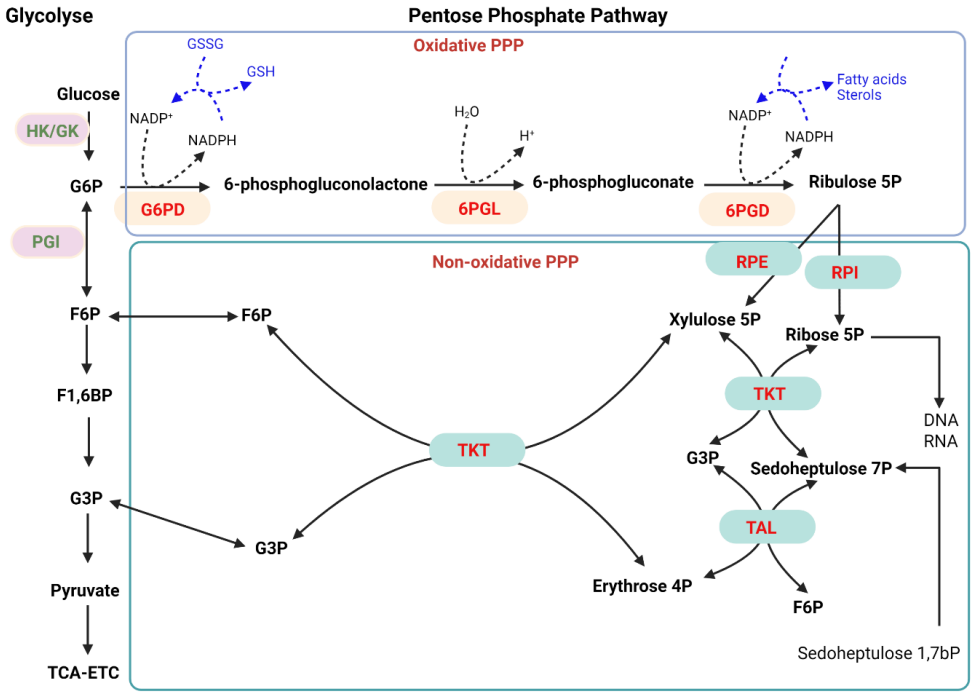



**Fig. (8.16). Schematic representation of the pentose phosphate pathway.** The PPP, also known as hexose phosphate shunt, branches after the first step of glycolysis and goes back to F6P and G3P in the glycolytic and gluconeogenic pathway. 6PGD, 6-phosphogluconate dehydrogenase; 6PGL, 6-phosphogluconolactonase; F6P, fructose 6-phosphate; F1,6BP, fructose 1,6-biphosphate; G3P, glyceraldehyde 3-phosphate; GK, glucokinase; $G_6PD$, glucose 6-phosphate dehydrogenase; HK, hexokinase; RPE, ribulose 5-phosphate epimerase; RPI, Ribose 5-phosphate isomerase; TAL, Taldo (transaldolase); TKT, transketolase.

It catalyzes the conversion of $G_6P$ to 6-phosphogluconolactone, accompanied by NADPH production. 6-phosphogluconolactone is then hydrolyzed by 6-phosphogluconolactonase to produce 6-phosphogluconate, which is converted to ribulose 5-phosphate with NAPDH generation by 6-phosphogluconate dehydrogenase. Several other enzymes, such as malic enzyme 1 (ME1) are also involved in the synthesis of cytosol NADPH. For the non-oxidative branch, ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase catalyze reversible reactions converting ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate, respectively. TKT catalyzes the conversion of xylulose 5-phosphate and ribose 5-phosphate to glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate, as well as the conversion of xylulose 5-phosphate and erythrose 4-phosphate to glyceraldehyde 3-phosphate and fructose-6-phosphate. The transaldolase reversibly converts glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate to erythrose 4-phosphate and fructose 6-phosphate. The non-

oxidative branch not only replenishes metabolites of the oxidative branch by the reversible reactions but also regulates the flux of glycolysis or gluconeogenesis by providing fructose-6-phosphate and glyceraldehyde 3-phosphate.

## CONCLUSION

Understanding the biochemistry of carbohydrates and the regulation of energetic molecules like glucose is a critical step to comprehending the pathophysiology of metabolic disorders. The regulation and retour of glucose is a complex process that involves many interconnected biochemical pathways. The objective of this chapter is to provide and summarize the current knowledge about carbohydrate metabolism and to facilitate the understanding of the underlying biochemical pathways.

## NOTES

[1] Ketosis is a metabolic state characterized by high levels of ketone bodies in the blood or urine.

[2] Anabolic pathways are metabolic pathways that construct (synthesis) molecules from smaller units.

[3] Catabolism is the breakdown of complex molecules to form simpler ones.

## REFERENCES

[1] Bhattacharya M. A historical exploration of Indian diets and a possible link to insulin resistance syndrome. Appetite 2015; 95: 421-54.
[http://dx.doi.org/10.1016/j.appet.2015.07.002] [PMID: 26206172]

[2] Jörgens V, Grüsser M. Happy Birthday, Claude Bernard. Diabetes 2013; 62(7): 2181-2.
[http://dx.doi.org/10.2337/db13-0700] [PMID: 23801718]

[3] Tanner ME. Understanding nature's strategies for enzyme-catalyzed racemization and epimerization. Acc Chem Res 2002; 35(4): 237-46.
[http://dx.doi.org/10.1021/ar000056y] [PMID: 11955052]

[4] Goodman BE. Insights into digestion and absorption of major nutrients in humans. Adv Physiol Educ 2010; 34(2): 44-53.
[http://dx.doi.org/10.1152/advan.00094.2009] [PMID: 20522896]

[5] Wright EM. The intestinal Na+/glucose cotransporter. Annu Rev Physiol 1993; 55(1): 575-89.
[http://dx.doi.org/10.1146/annurev.ph.55.030193.003043] [PMID: 8466186]

[6] Manolescu AR, Witkowska K, Kinnaird A, Cessford T, Cheeseman C. Facilitated hexose transporters: new perspectives on form and function. Physiology (Bethesda) 2007; 22(4): 234-40.
[http://dx.doi.org/10.1152/physiol.00011.2007] [PMID: 17699876]

[7] DeFina LF, Vega GL, Leonard D, Grundy SM. Fasting glucose, obesity, and metabolic syndrome as predictors of type 2 diabetes: the Cooper Center Longitudinal Study. J Investig Med 2012; 60(8): 1164-8.

[http://dx.doi.org/10.2310/JIM.0b013e318275656a] [PMID: 23111652]

[8] Jenkins DJ, Wolever TM, Taylor RH, *et al.* Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981; 34(3): 362-6.
[http://dx.doi.org/10.1093/ajcn/34.3.362] [PMID: 6259925]

[9] Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care 2008; 31(12): 2281-3.
[http://dx.doi.org/10.2337/dc08-1239] [PMID: 18835944]

[10] Bratanova-Tochkova TK, Cheng H, Daniel S, *et al.* Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion. Diabetes 2002; 51 (Suppl. 1): S83-90.
[http://dx.doi.org/10.2337/diabetes.51.2007.S83] [PMID: 11815463]

[11] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 2006; 7(2): 85-96.
[http://dx.doi.org/10.1038/nrm1837] [PMID: 16493415]

[12] Leney SE, Tavaré JM. The molecular basis of insulin-stimulated glucose uptake: signalling, trafficking and potential drug targets. J Endocrinol 2009; 203(1): 1-18.
[http://dx.doi.org/10.1677/JOE-09-0037] [PMID: 19389739]

[13] Unger RH, Aguilar-Parada E, Müller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest 1970; 49(4): 837-48.
[http://dx.doi.org/10.1172/JCI106297] [PMID: 4986215]

[14] Müller WA, Faloona GR, Aguilar-Parada E, Unger RH. Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. N Engl J Med 1970; 283(3): 109-15.
[http://dx.doi.org/10.1056/NEJM197007162830301] [PMID: 4912452]

[15] Gerich JE, Langlois M, Noacco C, Lorenzi M, Karam JH, Korsham PH. Comparison of the suppressive effects of elevated plasma glucose and free fatty acid levels on glucagon secretion in normal and insulin-dependent diabetic subjects. Evidence for selective alpha-cell insensitivity to glucose in diabetes mellitus. J Clin Invest 1976; 58(2): 320-5.
[http://dx.doi.org/10.1172/JCI108475] [PMID: 783198]

[16] Marliss EB, Aoki TT, Unger RH, Soeldner JS, Cahill GF Jr. Glucagon levels and metabolic effects in fasting man. J Clin Invest 1970; 49(12): 2256-70.
[http://dx.doi.org/10.1172/JCI106445] [PMID: 5480852]

[17] Bondy PK, Cardillo LR. The effect of glucagon on carbohydrate metabolism in normal human beings. J Clin Invest 1956; 35(5): 494-501.
[http://dx.doi.org/10.1172/JCI103302] [PMID: 13319484]

[18] Del Prato S, Castellino P, Simonson DC, DeFronzo RA. Hyperglucagonemia and insulin-mediated glucose metabolism. J Clin Invest 1987; 79(2): 547-56.
[http://dx.doi.org/10.1172/JCI112846] [PMID: 3543054]

[19] Unger RH. Glucagon physiology and pathophysiology in the light of new advances. Diabetologia 1985; 28(8): 574-8.
[http://dx.doi.org/10.1007/BF00281991] [PMID: 3902546]

[20] Jansson E, Hjemdahl P, Kaijser L. Epinephrine-induced changes in muscle carbohydrate metabolism during exercise in male subjects. J Appl Physiol 1986; 60: 1466-70.

[21] Guo R, Zong S, Wu M, Gu J, Yang M. Architecture of Human Mitochondrial Respiratory Megacomplex $I_2III_2IV_2$. Cell 2017; 170(6): 1247-1257.e12.
[http://dx.doi.org/10.1016/j.cell.2017.07.050] [PMID: 28844695]

[22] Iwata S, Lee JW, Okada K, *et al.* Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. Science 1998; 281(5373): 64-71.
[http://dx.doi.org/10.1126/science.281.5373.64] [PMID: 9651245]

[23] Vinothkumar KR, Zhu J, Hirst J. Architecture of mammalian respiratory complex I. Nature 2014; 515(7525): 80-4.
[http://dx.doi.org/10.1038/nature13686] [PMID: 25209663]

[24] Hunte C, Zickermann V, Brandt U. Functional modules and structural basis of conformational coupling in mitochondrial complex I. Science 2010; 329(5990): 448-51.
[http://dx.doi.org/10.1126/science.1191046] [PMID: 20595580]

[25] Wikström M, Hummer G. Stoichiometry of proton translocation by respiratory complex I and its mechanistic implications. Proc Natl Acad Sci USA 2012; 109(12): 4431-6.
[http://dx.doi.org/10.1073/pnas.1120949109] [PMID: 22392981]

[26] Ohnishi T. Piston drives a proton pump. Nature 2010; 465(7297): 428-9.
[http://dx.doi.org/10.1038/465428a] [PMID: 20505714]

[27] Sun F, Huo X, Zhai Y, *et al.* Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 2005; 121(7): 1043-57.
[http://dx.doi.org/10.1016/j.cell.2005.05.025] [PMID: 15989954]

[28] Yang XH, Trumpower BL. Purification of a three-subunit ubiquinol-cytochrome c oxidoreductase complex from Paracoccus denitrificans. J Biol Chem 1986; 261(26): 12282-9.
[http://dx.doi.org/10.1016/S0021-9258(18)67236-9] [PMID: 3017970]

[29] Tsukihara T, Aoyama H, Yamashita E, *et al.* The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. Science 1996; 272(5265): 1136-44.
[http://dx.doi.org/10.1126/science.272.5265.1136] [PMID: 8638158]

[30] Stumvoll M, Meyer C, Mitrakou A, Nadkarni V, Gerich JE. Renal glucose production and utilization: new aspects in humans. Diabetologia 1997; 40(7): 749-57.
[http://dx.doi.org/10.1007/s001250050745] [PMID: 9243094]

[31] Scott RB. The role of glycogen in blood cells. N Engl J Med 1968; 278(26): 1436-9.
[http://dx.doi.org/10.1056/NEJM196806272782607] [PMID: 4875345]

[32] Oe Y, Baba O, Ashida H, Nakamura KC, Hirase H. Glycogen distribution in the microwave□fixed mouse brain reveals heterogeneous astrocytic patterns. Glia 2016; 64(9): 1532-45.
[http://dx.doi.org/10.1002/glia.23020] [PMID: 27353480]

[33] Dean M. Glycogen in the uterus and fallopian tubes is an important source of glucose during early pregnancy. Biol Reprod 2019; 101(2): 297-305.
[http://dx.doi.org/10.1093/biolre/ioz102] [PMID: 31201425]

[34] Adeva-Andany MM, González-Lucán M, Donapetry-García C, Fernández-Fernández C, Ameneiros-Rodríguez E. Glycogen metabolism in humans. BBA Clin 2016; 5: 85-100.
[http://dx.doi.org/10.1016/j.bbacli.2016.02.001] [PMID: 27051594]

[35] Heikinheimo P, Lehtonen J, Baykov A, Lahti R, Cooperman BS, Goldman A. The structural basis for pyrophosphatase catalysis. Structure 1996; 4(12): 1491-508.
[http://dx.doi.org/10.1016/S0969-2126(96)00155-4] [PMID: 8994974]

[36] Lomako J, Lomako WM, Whelan WJ. Glycogenin: the primer for mammalian and yeast glycogen synthesis. Biochim Biophys Acta, Gen Subj 2004; 1673(1-2): 45-55.
[http://dx.doi.org/10.1016/j.bbagen.2004.03.017] [PMID: 15238248]

[37] Pederson BA, Cheng C, Wilson WA, Roach PJ. Regulation of glycogen synthase. Identification of residues involved in regulation by the allosteric ligand glucose-6-P and by phosphorylation. J Biol Chem 2000; 275(36): 27753-61.
[http://dx.doi.org/10.1016/S0021-9258(19)65048-9] [PMID: 10874034]

[38] Chen YT, He JK, Ding JH, Brown BI. Glycogen debranching enzyme: purification, antibody characterization, and immunoblot analyses of type III glycogen storage disease. Am J Hum Genet 1987; 41(6): 1002-15.

[PMID: 2961257]

[39] Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. Nat Rev Endocrinol 2017; 13(10): 572-87.
[http://dx.doi.org/10.1038/nrendo.2017.80] [PMID: 28731034]

[40] Cross DAE, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995; 378(6559): 785-9.
[http://dx.doi.org/10.1038/378785a0] [PMID: 8524413]

[41] Roden M, Perseghin G, Petersen KF, *et al.* The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. J Clin Invest 1996; 97(3): 642-8.
[http://dx.doi.org/10.1172/JCI118460] [PMID: 8609218]

[42] Bates S, Gardiner J, Jones R, Bloom S, Bailey C. Acute stimulation of glucose uptake by leptin in l6 muscle cells. Horm Metab Res 2002; 34(3): 111-5.
[http://dx.doi.org/10.1055/s-2002-23192] [PMID: 11972298]

[43] Berti L, Kellerer M, Capp E, Häring HU. Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. Diabetologia 1997; 40(5): 606-9.
[http://dx.doi.org/10.1007/s001250050722] [PMID: 9165231]

[44] Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, Häring HU. Leptin activates PI-3 kinase in C2C12 myotubes *via* janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. Diabetologia 1997; 40(11): 1358-62.
[http://dx.doi.org/10.1007/s001250050832] [PMID: 9389430]

[45] Roach PJ, Depaoli-Roach AA, Hurley TD, Tagliabracci VS. Glycogen and its metabolism: some new developments and old themes. Biochem J 2012; 441(3): 763-87.
[http://dx.doi.org/10.1042/BJ20111416] [PMID: 22248338]

[46] Jensen J, Rustad PI, Kolnes AJ, Lai YC. The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Front Physiol 2011; 2: 112.
[http://dx.doi.org/10.3389/fphys.2011.00112] [PMID: 22232606]

[47] Stincone A, Prigione A, Cramer T, *et al.* The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. Biol Rev Camb Philos Soc 2015; 90(3): 927-63.
[http://dx.doi.org/10.1111/brv.12140] [PMID: 25243985]

[48] Horecker BL. The pentose phosphate pathway. J Biol Chem 2002; 277(50): 47965-71.
[http://dx.doi.org/10.1074/jbc.X200007200] [PMID: 12403765]

[49] Chen L, Zhang Z, Hoshino A, *et al.* NADPH production by the oxidative pentose-phosphate pathway supports folate metabolism. Nat Metab 2019; 1(3): 404-15.
[http://dx.doi.org/10.1038/s42255-019-0043-x] [PMID: 31058257]

[50] Yen WC, Wu YH, Wu CC, *et al.* Impaired inflammasome activation and bacterial clearance in $G_6$PD deficiency due to defective NOX/p38 MAPK/AP-1 redox signaling. Redox Biol 2020; 28: 101363.
[http://dx.doi.org/10.1016/j.redox.2019.101363] [PMID: 31707353]

# CHAPTER 9

# Lipid Metabolism

**Abstract:** Lipids have many functional roles such as bilayer of the cell membrane, regulatory agents in cell growth, adhesion, and the biosynthesis of other molecules. Lipid metabolism starts with the ingestion of food, digestion and absorption. In higher organisms, fat metabolism is under the control of a complex and highly integrated system and pathways. The present chapter aims to provide current knowledge about lipid metabolism and its cellular processes.

**Keywords:** Cholesterol, Fatty acids, Lipid metabolism, Lipoproteins, Lipolysis, Oxidation, Synthesis, Ketogenesis.

## INTRODUCTION

Generally, lipids are defined as insoluble substances in water but soluble in organic solvents (acetone, chloroform, ether). There are different types of lipids, including fatty acids, triglycerides (also known as triacylglycerols), phospholipids, and cholesterols (esters). In the body, the lipids are obtained from diets (30% or more of the total energy consumed, see please chapter 1 for the diet composition) or *de novo* synthesized. During fed state and for an average body weight adult male, fats are stored as triglycerides as much as 90,000-100,000 Kcal. During fasting, fats are oxidized to produce ATP and, therefore, are considered the main source of energy during starvation. Some lipids, such as steroids (steroid hormones) serve as chemical messengers and signaling pathways between cells. Phospholipids are components of cell membranes, which function to separate individual cells from their environments. In this chapter, I will describe the general properties and classes of lipids, discuss their digestion and absorption, and finish the chapter with the fat metabolism during synthesis and degradation and their underlying molecular mechanisms.

### 9.1. Classification of Lipids

Lipids can be classified as saponifiable and nonsaponifiable lipids. Saponifiable lipids are made up of long chain carboxylic (fatty) acids connected to an alcoholic functional group via the ester linkage, which can undergo saponification and

hydrolysis in the presence of base, acid, or enzymes (Fig. **9.1a**). Triglycerides, phospholipids, waxes, and sphingolipids are saponifiable lipids. Nonsaponifiable lipids, such as cholesterol and prostaglandine, can be degraded via hydrolysis into smaller molecules. Further, these lipids can be divided into polar and nonpolar lipids. Polar lipids are amphiphilic lipids with a hydrophilic head and a hydrophobic tail and are often associated with membrane structure, such as glycerophospholipids [1]. Nonpolar lipids have no charged area and are hydrophobic, such as triglycerides. Specifically, lipids can also be divided into simple and complex lipids. Simple lipids, such as waxes, contain esters of fatty acids with higher molecular weight monohydric alcohols. Complex lipids, however, contain not only fatty acid and alcohol but also additional groups. For instance, phospholipids contain in addition to fatty acids and alcohol, a phosphoric acid residue. Glycolipids, such as glycosphingolipids, contain fatty acids, sphingosine, and carbohydrate. Among the precursors and derived lipids, we found fatty acids, glycerol, steroids, alcohols, fatty aldehydes, ketone bodies, hydrocarbons, vitamins (absorbed in lipid micelles such as vitamin A, D, E, and k).

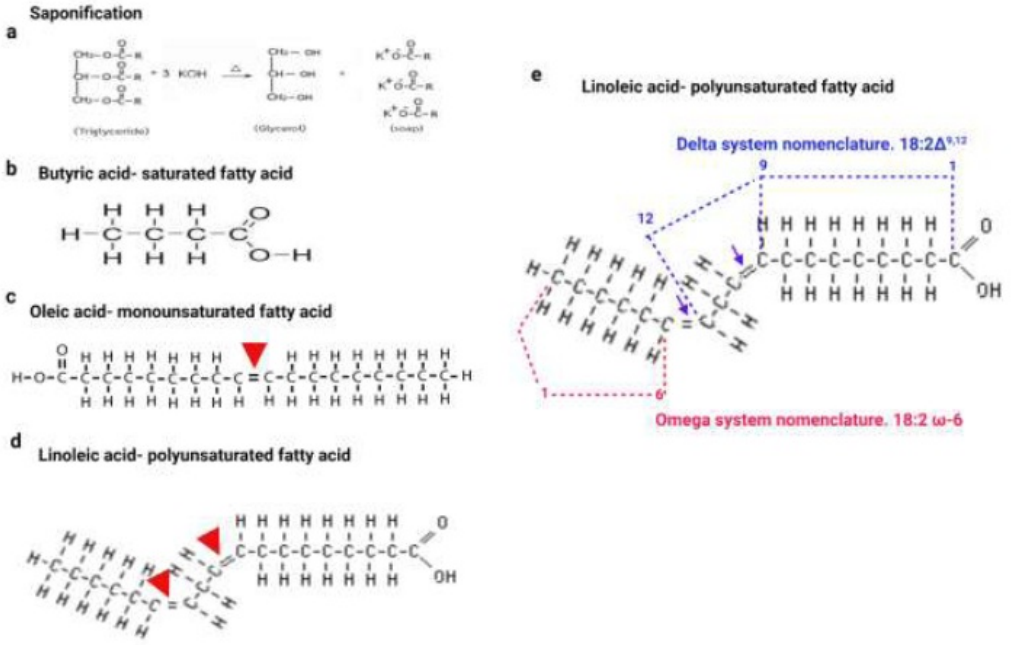



**Fig. (9.1). Structure of saturated and unsaturated fatty acids and their nomenclatures. a)** saponification reaction, **b)** butyric acid, **c)** oleic acid, **d)** linoleic acid, and **2)** Delta and omega system nomenclatures.

### *9.1.1. Fatty Acids*

Fatty acids are organic (carboxylic) acids, usually with a polar carboxyl end and nonpolar methyl end, and the two ends are separated by a hydrocarbon region of varying lengths. The chain length is determined by the total number of carbons. For instance, acetic acid has only two carbons and hence it is the shortest fatty acid. Arachidic acid, on the other hand, has 20 carbons, and it is one of the longest fatty acids. Based on the length of the hydrocarbon region, fatty acids are sub-classified into short-chain (2-4 carbons), medium-chain (6-12 carbons), and long-chain (14-26 carbons) (Table **9.1**). Beyond length, fatty acids can contain double bonds between carbon-carbon molecules and are sub-classified to saturated fatty acid (SFA, no carbon-carbon double bond, such as butyric acid (Fig. **9.1b**). Monounsaturated fatty acids (MUFAs) contain a single carbon-carbon double bond, such as oleic acid (Fig. **9.1c**). Polyunsaturated fatty acids (PUFA) contain more than one carbon-carbon double bond, such as linoleic acid (Fig. **9.1d**).

**Table 9.1. Fatty acid classification and nomenclature.**

| Fatty Acids | C Atoms | Double Bonds | ω System |
|---|---|---|---|
| **Saturated** | | | |
| Acetic acid | 2 | 0 | C2:0 |
| Butyric acid | 4 | 0 | C4:0 |
| Caproic acid | 6 | 0 | C6:0 |
| Caprylic acid | 8 | 0 | C8:0 |
| Capric acid | 10 | 0 | C10:0 |
| Lauric acid | 12 | 0 | C12:0 |
| Myristic acid | 14 | 0 | C14:0 |
| Palmitic acid | 16 | 0 | C16:0 |
| Stearic acid | 18 | 0 | C18:0 |
| Arachidic acid | 20 | 0 | C20:0 |
| Behenic acid | 22 | 0 | C22:0 |
| Lignoceric acid | 24 | 0 | C24:0 |
| **Monounsaturated** | | | |
| Palmitoleic acid | 16 | 1 | C16:1 ω7 |
| Oleic acid | 18 | 1 | C18:1 ω9 |
| Cetoleic acid | 22 | 1 | C22:1 ω11 |
| Nervonic acid | 24 | 1 | C24:1 ω9 |
| **Polyunsaturated** | | | |

*(Table 1) cont.....*

| | | | |
|---|---|---|---|
| Linoleic acid | 18 | 2 | C18:2 ω6 |
| α-Linolenic acid | 18 | 3 | C18:3 ω3 |
| γ-Linolenic acid | 18 | 3 | C18:3 ω6 |
| Arachdonic acid | 20 | 4 | C20:4 ω6 |
| Eicosapentaenoic acid | 20 | 5 | C20:5 ω3 |
| Docosatetraenoic acid | 22 | 4 | C22:4 ω6 |
| Docosapentaenoic acid | 22 | 5 | C22:5 ω3 |
| Docosahexaenoic acid | 22 | 6 | C22:6 ω3 |

For the nomenclature, the position of double bonds can be accomplished by using the delta (Δ) system, which consists of counting the carbon number in the chain from the carboxyl (alpha) end. For example, linoleic acid contains 18 carbons, with 2 carbon-carbon bonds at position 9 and 12, thus it is $18:2\Delta^{9,12}$. Using the omega (the last letter of the Greek alphabet) system, which consists of counting the carbon number from the methyl end, the nomenclature of linoleic acid, for example, is 18:2 ω-6 or sometimes the n letter is used for substitution (18:2 n-6) (Fig. **9.1e**).

Unsaturated fats can be either in the cis or trans configuration. In the cis configuration, the chains of carbon atoms are on the same side of the double bond, and hydrogens are present in the same plane, resulting in a kink that prevents the fatty acids from packing tightly. In the trans arrangement, however, the hydrogen atoms are on two different planes (Fig. **9.2**). For example, oleic acid can have two configurations: cis, called oleic acid, and trans, called elaidic acid [2].

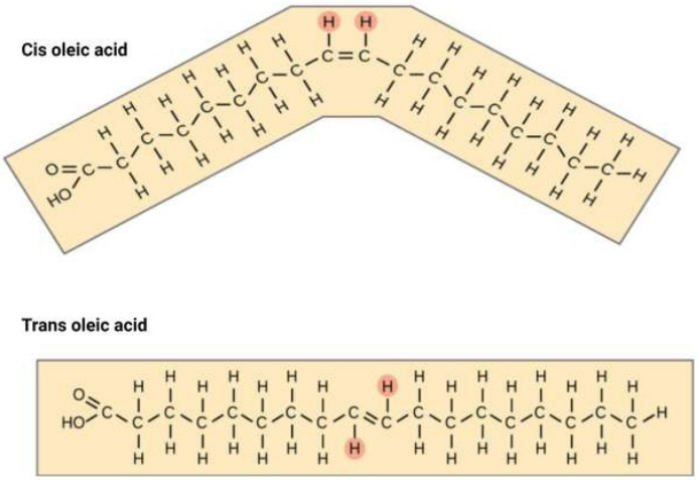



**Fig. (9.2).** Cis *vs.* trans arrangement of linoleic acid.

### *9.1.2. Essential Fatty Acids*

Essential fatty acids are fatty acids required for biological processes but not synthesized by the body, and therefore have to be supplemented through ingestion via the diet and are nutritionally very important. Humans do not have Δ-12 and Δ-15 desaturases, and thus 18 C-ω3 and 18 C-ω6 PUFA are dietary essential for humans. Alpha-linoleic acid (ALA, 18:2 ω-6) and linolenic acid (18:3 ω-3) are the two PUFAs fall into this category. Research indicates that these essential fatty acids reduce the risk of sudden death from heart attacks, reduce triglycerides in the blood, lower blood pressure, and prevent thrombosis by inhibiting blood clotting. They also reduce inflammation and may help reduce the risk of some cancers in animals [3 - 7].

The other long-chain PUFA, including arachidonic acid (AA, C20:4 ω-6), eicosapentaenoic acid (EPA, C20:5 ω-3), and docosahexaenoic acid (DHA, C22:6 ω-3) can be generated from available linoleic and linolenic acids by a series of elongation and desaturations (Fig. **9.3**).

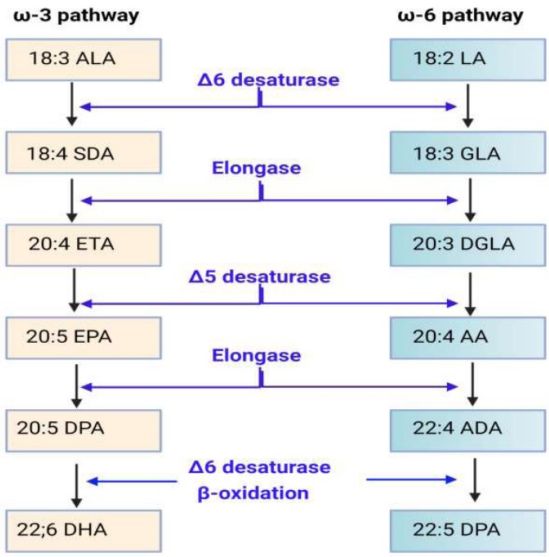



**Fig. (9.3). PUFA synthetic pathways.** α -linolenic acid (ALA; 18:3ω-3) and linoleic acid (LA; 18:2 ω-6) are essential PUFA obtained from the diet and are substrates for elongase and desaturases for the synthesis of long chain, more unsaturated PUFA eicosapentaenoic acid (EPA; 20:5 ω-3), docosahexaenoic acid (DHA; 22:6 ω-3), and arachidonic acid (AA; 20:4 ω-6). Relevant intermediates in these pathways include SDA (stearidonic acid), ETA (eicosatetraenoic acid), DPA (docosapentaenoic acid), GLA (γ -linolenic acid), DGLA (dihomo- γ -linolenic acid), and ADA (adrenic acid). These pathways employ the same enzymes, however, they are not interconvertible in mammals (ω-6 PUFA cannot be converted into ω-3 PUFA and vice versa). The products of ω-6 PUFA tend to exert inflammatory effects, while the products of ω-3 PUFA tend to be anti-inflammatory.

### *9.1.3. Triglycerides*

Triglycerides, also known as triacylglycerols, are esters derived from glycerol and three fatty acids (Fig. **9.4**). They are the major form of fat in both food and the body. There are also monoacylglycerol and diacylglycerol where the glycerol is attached to one or two fatty acids, respectively. If the fatty acids are more saturated, thus the triglycerides are likely to be solid at room temperature. However, triglycerides with more unsaturated fatty acids or short chain-fatty acids are likely to be liquid.

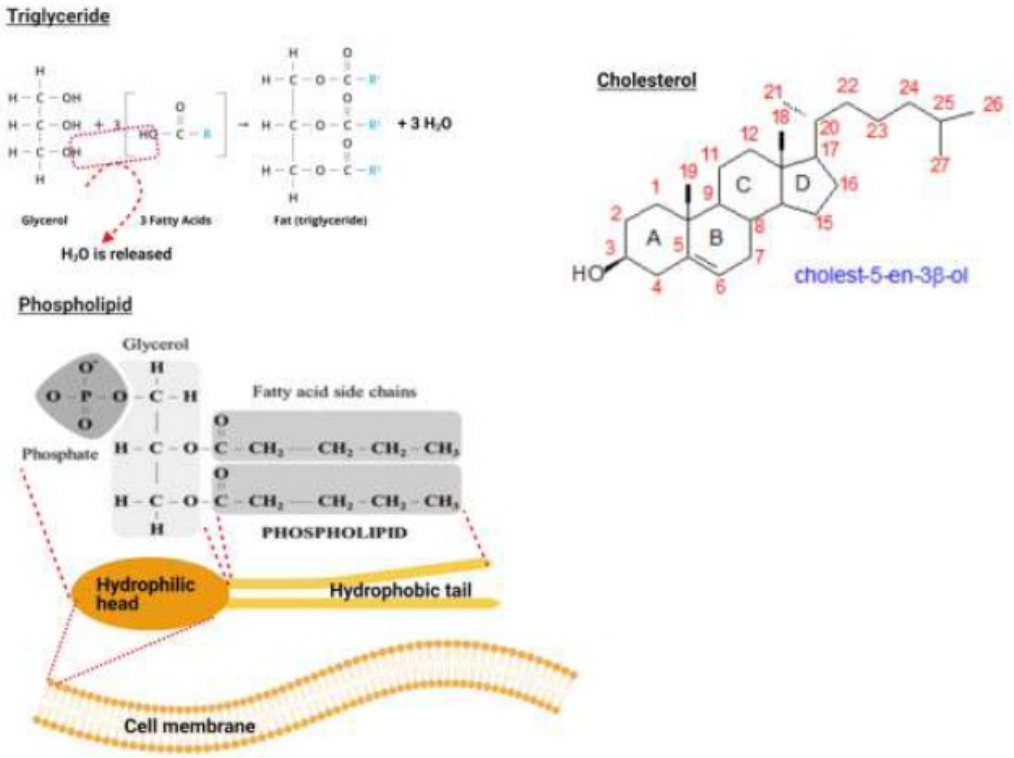



**Fig. (9.4). Structure of triglyceride, phospholipids, and cholesterol.** The R in each fatty acid molecule represents the long carbon chain, and it may or may not be the same.

### *9.1.4. Phospholipids*

Phospholipids contain phosphatidic acid, which is composed of glycerol, two fatty acids, and a phosphate ($PO_4$) group (Fig. **9.4**). The alpha carbon is usually esterified to a saturated fatty acid, and the beta carbon is esterified to an unsaturated fatty acid. Several phospholipids, including phosphatidylcholine,

phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, are of nutritional and physiologic relevance. For instance, phosphatidylinositol acts as a source of inositol triphosphate and diacylglycerol in the membrane produced as intracellular second messengers in response to neurotransmitters. Another example is phosphatidylcholines, which are synthesized in the endoplasmic reticulum, where they have essential functions, including the provision of membranes required for protein synthesis and export, cholesterol homeostasis, and triacylglycerol storage and secretion [8].

### *9.1.5. Cholesterols and Steroids*

Cholesterol, from the Greek word "chole-bile; stereos-solid, and ol-alcohol", cholest-5-en-3β-ol) is by far the most abundant member of a family of polycyclic compounds known as sterol. Cholesterol was first recognized in 1769 as a component of gallstones, while the French lipid chemist Michel Eugène Chevreul isolated it from animal fats in 1815. The structure of cholesterol (Fig. **9.4**) was defined in the 20$^{th}$ century by the German Chemist Heinrich Wieland, who received the Nobel Prize in Chemistry in 1927. Cholesterol is a ubiquitous component of all animal tissues where much of it is located in the membranes. The highest proportion of unesterified cholesterol is in the plasma membrane (roughly 30-50% of the lipid in the membrane or 60-80% of the cholesterol in the cell), while mitochondria and the endoplasmic reticulum have much less (~5%), and the Golgi contains an intermediate amount. Cholesterol is also enriched in the brain and early and recycling endosomes [9]. Animals in general synthesize a high proportion of their cholesterol requirement, but they can also ingest and absorb appreciable amounts from foods (200-500 mg/day). Cholesterol is a precursor for steroid hormones such as estrogens (17-β-estradiol), androgens, cortisol, aldosterone, as well as vitamin D and bile salts [10 - 13].

## 9.2. Digestion and Absorption of Lipids

The digestion of dietary lipids begins in the mouth. Chewing mechanically breaks food into smaller particles and mixes them with saliva. A lipase, lingual lipase, is secreted by the tongue starts the enzymatic digestion cleaving individual fatty acids from the glycerol backbone [14], but this digestion is only marginal. Fat hydrolysis continues in the stomach, where a gastric lipase is secreted and results in the liberation of free fatty acids and two monoacylglycerols. Most of the dietary lipids are clustered in the stomach to form a coarse emulsion of chyme[1], which then enters the duodenum, the first section of the small intestine (Fig. **9.5**).

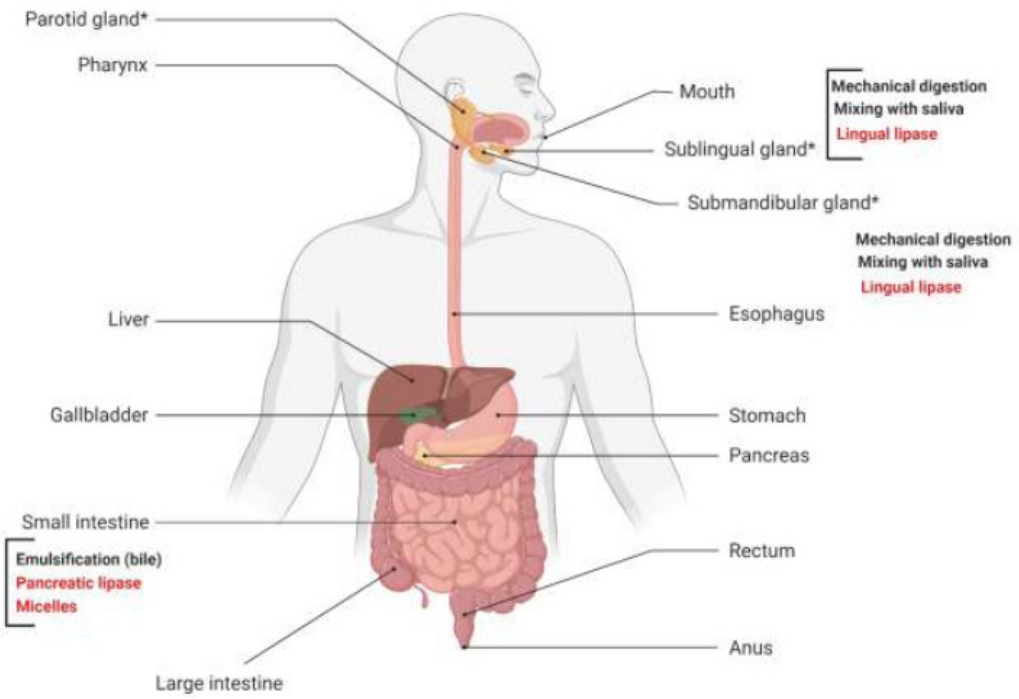



**Fig. (9.5).** Schematic representation of lipid digestion.

This, in turn, triggers the release of bile, which is produced in the liver and stored in the gallbladder and facilitates the emulsification[2] process. Bile contains bile salts, which have hydrophobic and hydrophilic sides. The bile salts' hydrophilic side can interface with water, while the hydrophobic side interfaces with lipids, thereby emulsifying large lipid globules into small lipid globules. As emulsification proceeds, the pancreatic lipase hydrolyses the lipids and further enhances the emulsification process and the formation of micelles[3]. The pancreatic lipase is under the control of CCK, which also regulates gallbladder relaxation and bile release. An additional enzyme, the pancreatic colipase, acts on the micelle surface and hydrolyzes triglycerides. As a result of lipase activities, monoglycerides, fatty acids, and glycerol are released into the aqueous environment of the intestinal lumen and are continually solubilized by the bile salts. Cholesterol and phospholipids are digested by cholesterol esterase and phospholipases, respectively [15].

The end products of digested lipids (free fatty acids and monoacylglycerols from triglycerides, free fatty acids and lysophospholipids from phospholipids, and free fatty acids and free cholesterols from cholesterol esters) enter in contact with the surface of the microvilli. Enterocytes use diffusion and protein-mediated transport

mechanisms to take up monoacylglycerols and free fatty acids [16]. The diffusion transport occurs when the concentration of free fatty acids in the lumen exceeds that inside the epithelial cells. When the extracellular concentrations of free fatty acids are lower, the protein-mediated transport mechanism is activated and becomes dominant (Fig. **9.6**). Several proteins, including fatty acid transport proteins (FATPs) and the cluster of differentiation (CD36), have been shown to be involved in this process [17]. Once they enter the epithelial cell, free fatty acids are further transported to various organelles for further processing by fatty acid-binding proteins (FABPs) [18].

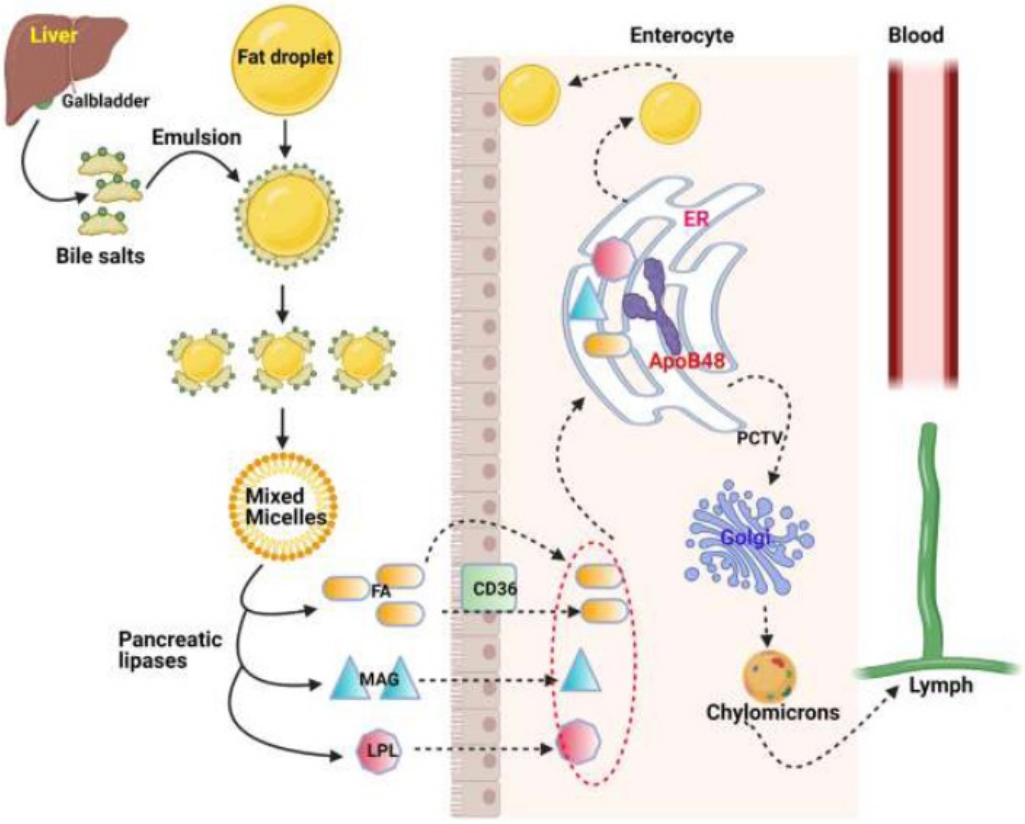



**Fig. (9.6). Schematic representation of intestinal lipid absorption.** Dietary lipids are emulsified with bile salts and digested with pancreatic lipases, generating free fatty acids, monoacylglycerols, and free cholesterols. These products enter the enterocyte by various mechanisms (diffusion of protein-mediated transport) and reach the reticulum endoplasmic, where they are used to synthesize triacylglycerols, phospholipids, and cholesterols esters. Next, these lipids are assembled in chylomicrons using apoB48 and they are either stored in the cytosol as lipid droplets or secreted via cis Golgi into the lymph system. CD36, a cluster of differentiation 36; ER, endoplasmic reticulum; FA, fatty acid; MAG, monoacylglycerol.

Monoacylglycerols are transported to the endoplasmic reticulum where they are esterified with free fatty acids by monoacylglycerol acyltransferases to form diacylglycerols, which in turn are converted to triacylglycerides by diacylglycerol acyltransferases [19]. Diacylglycerols can also be associated with choline and ethanolamine to produce phospholipids through choline transferases and ethanolamine transferases. Endoplasmic reticulum cholesterols are esterified by membrane-bound acyl-CoA/cholesterol acyltransferase to form cholesteryl esters and glycerolipids.

The synthesized lipids in the enterocyte endoplasmic reticulum have two fates: 1) they can become part of cytosolic lipid droplets, which contain a core (neutral triglycerides and cholesterol esters), surrounded by phospholipid monolayers [17, 20 - 22]. During the energy depletion and fasting period, the enterocyte cytosol lipid droplets are hydrolyzed, and the free fatty acids enter the endoplasmic reticulum to re-synthetize triglycerides for subsequent secretion with lipoproteins. 2) The enterocyte endoplasmic reticulum lipids can be secreted. First, lipids are packaged into large spherical triacylglycerol-rich lipoproteins, called chylomicrons (from the Greek word, chylos-juice and micron-small particle). The chylomicron core is rich in triacylglycerol (85-92%) and cholesterol esters (1-3%). The chylomicron surface is covered with phospholipid monolayer (6-125) and is surrounded by a large nonexchangeable protein, apolipoprotein B48 (Apob48), and several exchangeable proteins such as apoAI, apoAIV, and apoCs. The apoB48-containing lipoproteins are synthesized in the intestine [23 - 25]. Chylomicron assembly begins with the translation of apoB48, which is folded into a structural configuration that is receptive to accepting more lipids and results in the formation of high-density lipoprotein (HDL), followed by a large bolus of lipids to produce larger lipoproteins [26]. Chylomicron particles are transported by pre-chylomicron transport vesicles (PCTVs) and fuse with the cis-Golgi via the coat protein complex II-interacting proteins [27 - 29]. Chylomicrons undergo further modification in cis-Golgi, such as glycosylation of apoB48, and then released from the basolateral membrane into the lacteals, where they join lymph to become chyle. The lymphatic vessels carry the chyle to the venous return of the systemic circulation. Chylomicrons bypass the hepatic portal system and supply tissues with fat absorbed from the diet.

## 9.3. Lipoprotein Metabolism

Lipoproteins are primarily produced in the enterocytes and hepatocytes. The core of a typical lipoprotein is composed of cholesterol esters and triglycerides and may be some diacylglycerols and monoacylglycerols. The main types of lipoproteins are chylomicrons (previous section), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

### *9.3.1. VLDL Metabolism*

VLDLs are produced in the liver, where triglycerides and cholesterol esters are transferred in the endoplasmic reticulum to newly synthesized ApoB-100 [30]. The rate of hepatic VLDL synthesis depends on the availability of triglycerides. The microsomal triglyceride transfer protein (MTP) is required for the early transfer of lipid to apoB-100, but not for the additional lipid transfer. Secreted VLDL transports endogenously produced triglycerides and cholesterols as well as that derived from chylomicrons to peripheral tissues such as adipose tissue and muscle [30]. The hydrolysis of VLDL (triglycerides) by lipoprotein lipase on the muscle and adipose tissue releases fatty acids that are uptaken by target tissues and generates intermediate-density lipoprotein (IDL) or VLDL remnants, which are enriched in cholesterol esters. LDL particles can be removed from the circulation by the liver via binding of Apo E to LDL and LDL receptor (Fig. **9.7**), and the remnants IDL are hydrolyzed by hepatic lipase leading to a decrease in circulating triglycerides levels and exchangeable apolipoproteins are transferred from IDL to other lipoproteins resulting in LDL formation.

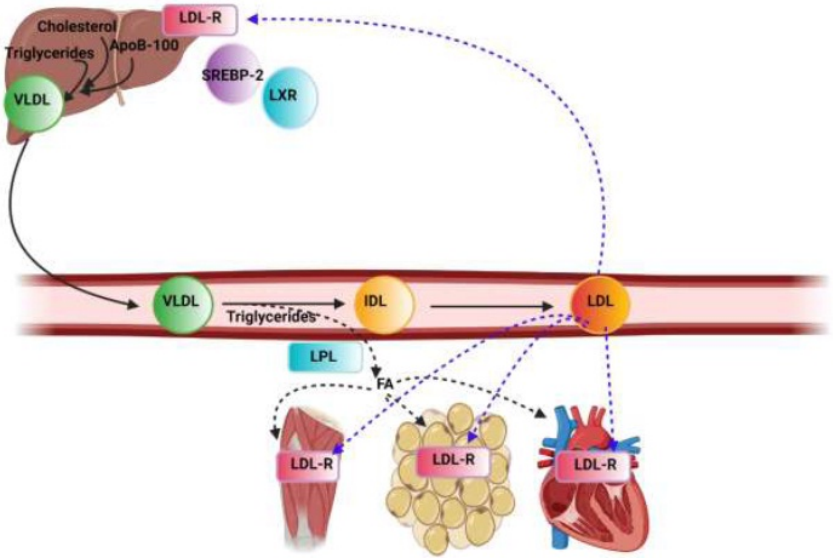



**Fig. (9.7). Schematic representation of intestinal lipid absorption.** Dietary lipids are emulsified with bile salts and are hydrolyzed by different pancreatic lipases resulting in the generation of free fatty acids, monoacylglycerols, and free cholesterol. The digested lipids are taken up by the enterocytes via different transporters and reach the endoplasmic reticulum where they are used to synthesize phospholipids, triacylglycerols, and cholesterol esters. Next, these lipids are assembled into chylomicron particles using apoB48 and have two fates: 1) they are stored in the cytosol as lipid droplets or 2) secreted in the lymph. ER, endoplasmic reticulum; FA, fatty acids, LPL, lipoprotein lipase; MAG, monoacylglycerol; PCTV, pre-chylomicron transport vesicles.

### *9.3.2. LDL Metabolism*

Plasma levels of LDL depends on the number of hepatic LDL receptor and LDL production rate. Approximately 70% of circulating LDL is cleared via hepatocyte LDL receptor mediated endocytosis, with the remainder taken up by extrahepatic tissues. The hepatic LDL receptor is also regulated by the hepatic cholesterol levels, which are under the control of sterol regulatory element binding protein 2 (SREBP2). In fact, SREBP2 is transported from the endoplasmic reticulum to the Golgi, where proteases cleave the SREBP2 into active SREBP2, which translocate in the nucleus and induce LDL receptor expression and hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol synthesis. Additionally, liver X receptor (LXR) activates the E3 ubiquitin ligase that mediates the ubiquitination and degradation of LDL receptor [31, 32] (Fig. **9.7**).

### *9.3.3. HDL Metabolism*

ApoA-I is synthesized and secreted from the liver and intestine, and assembled with cholesterol esters and phospholipids to form HDL via ATP-binding cassette transporter 1 (ABCA1), also known as cholesterol efflux regulatory protein (CERP). Free cholesterol and phospholipids can be obtained from the liver, intestine and other peripheral tissues, as well as from VLDL hydrolysis and chylomicrons. For esterification, lecithin-cholesterol acyltransferase-like 1 (LCAT1) catalyzes the transfer of fatty acid from phospholipids to free cholesterol, forming cholesterol esters that constitute the core of HDL. This core cholesterol ester can be transferred to ApoB in exchange for triglycerides via cholesterol ester transfer protein (CEPT), resulting in triglyceride-rich HDL. This HDL can be hydrolyzed by hepatic lipase, resulting in the formation of small HDL. The circulating HDL binds to the HDL receptor scavenger receptor class B type I (SR-BI) on the liver surface, which allow the cholesterol from HDL to be transported into the liver without internalization of HDL particles [33].

### *9.3.4. Cholesterol Metabolism and Reverse Cholesterol Transport*

Peripheral cells accumulate cholesterols via the uptake of lipoproteins and *de novo* cholesterol biosynthesis, which involves a highly complex series of different enzymatic reactions. More than 30 enzymatic reactions are involved, defined in large measure by the Polish scientist Konrad Bloch and the German scientist Feodor Lynen, who received the Nobel Prize in Physiology and Medicine in 1964. As depicted in (Fig. **9.8**), cholesterol biosynthesis starts with the synthesis of

mevalonic acid from acetyl-CoA and acetoacetyl-CoA, derivatives of acetate, by 3-hydroxy-3-methyl-glutaryl(HMG)-CoA synthase and HMG-CoA reductase. In the next step of the synthesis, mevalonic acid is phosphorylated by mevalonate kinase to form the 5-monophosphate ester, followed by further phosphorylation to yield an unstable

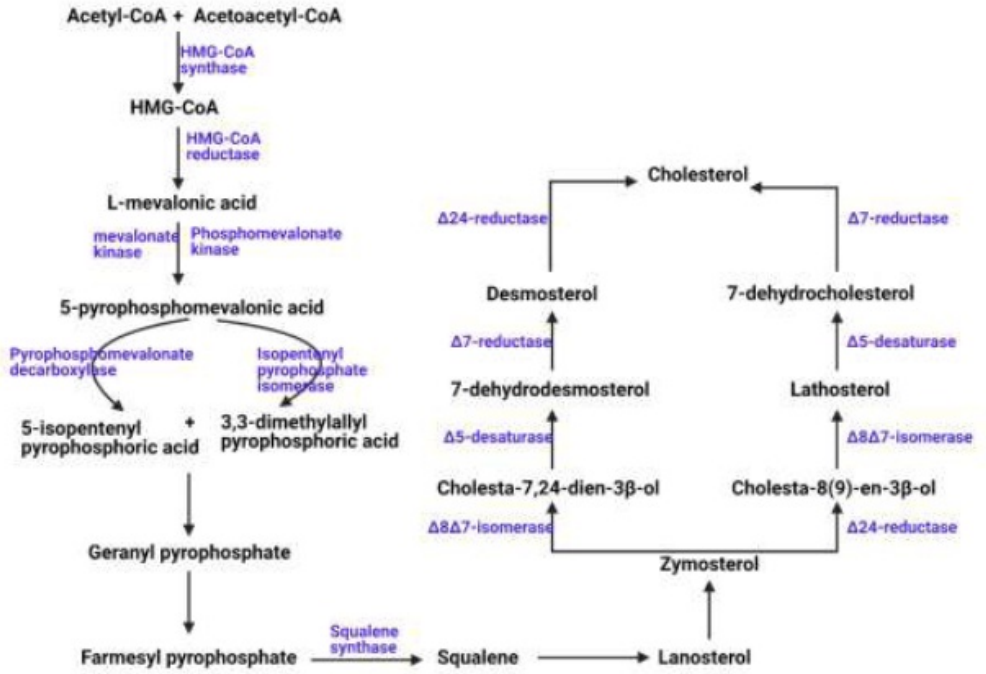



**Fig. (9.8). Schematic representation of cholesterol biosynthesis.** HMG, 3-hydroxy-3-methylglutaryl.

pyrophosphate, which is rapidly decarboxylated to produce 5-isopentenyl pyrophosphoric acid, the universal isoprene unit. An isomerase converts part of the latter to 3,3-dimethylallyl pyrophosphoric acid. Next, 5-isopentenyl pyrophosphate and 3,3-dimethylallyl pyrophosphate condense with the elimination of pyrophosphoric acid to form the monoterpenoid derivative geranyl pyrophosphate. This reacts further with another molecule of 5-isopentenyl pyrophosphate to produce the sesquiterpene derivative (C15) farnesyl pyrophosphate, two molecules of which are condensed to yield presqualene pyrophosphate. This is reduced, next, by NADPH and produces squalene by squalene synthase. In the next step, squalene is oxidized by a squalene monooxygenase to squalene 2,3-epoxide, a key control point in the cholesterol synthesis pathway. Oxygen atom is introduced in squalene to form the hydroxyl group in cholesterol. The squalene epoxide lanosterol-cyclase catalyzes the

epoxide cyclization resulting in the formation of lanosterol. This reaction occurs in the reticulum endoplasmic in the presence of NADPH, FAD, and $O_2$. Several reactions of demethylations, desaturations, isomerizations, and reductions convert lanosterol to cholesterol.

Most of the cells, except the liver, do not have a mechanism to degrade cholesterol. Cells that synthesize steroid hormones can convert cholesterol to estrogen, androgen, *etc*. Only the liver possesses the enzymes to degrade significant amounts of cholesterol and form oxysterol and bile acid. Cholesterols are transferred back from peripheral tissues in lipoprotein complexes to the liver for catabolism.

## 9.4. Fatty Acid Synthesis

The liver, and to a lesser degree, the adipose tissue, is the main site for *de novo* fatty acid synthesis[4], although some other tissues such as the kidney, mammary gland, lungs, and the brain have the ability to synthesize fatty acids. Fatty acid synthesis occurs in the cytosol. In fed state and abundant intracellular energy, the fatty acid synthesis starts with the carboxylation of acetyl-CoA to malonyl CoA via acetyl-CoA carboxylase (ACC) [34, 35], which is an irreversible reaction and the committed step in the fatty acid synthesis. ACCs are large and homodimeric multienzymes that exist in two isoforms: the metabolic, cytosolic ACC1 or ACCα, and mitochondrial-associated ACC2 (ACCβ) [36, 37]. This reaction needs biotin and the hydrolysis of ATP molecule. The enzyme system that catalyzes the synthesis of saturated long-chain fatty acids from acetyl CoA, malonyl CoA and NADPH is called fatty acid synthase (FAS) [38 - 40]. Mammalian fatty acid synthase is a dimer of identical 260-kD subunits. As shown in (Fig. **9.9**), each chain is folded into three domains joined by flexible regions, and all seven different catalytic sites are present on a single polypeptide chain:

- Domain 1, the substrate entry and condensation unit, contains acetyl transferase, malonyl transferase, and β-ketoacyl synthase (condensing enzyme).

- Domain 2, the reduction unit, contains the acyl carrier protein, β-ketoacyl reductase, dehydratase, and enoyl reductase.

- Domain 3, the palmitate release unit, contains the thioesterase.

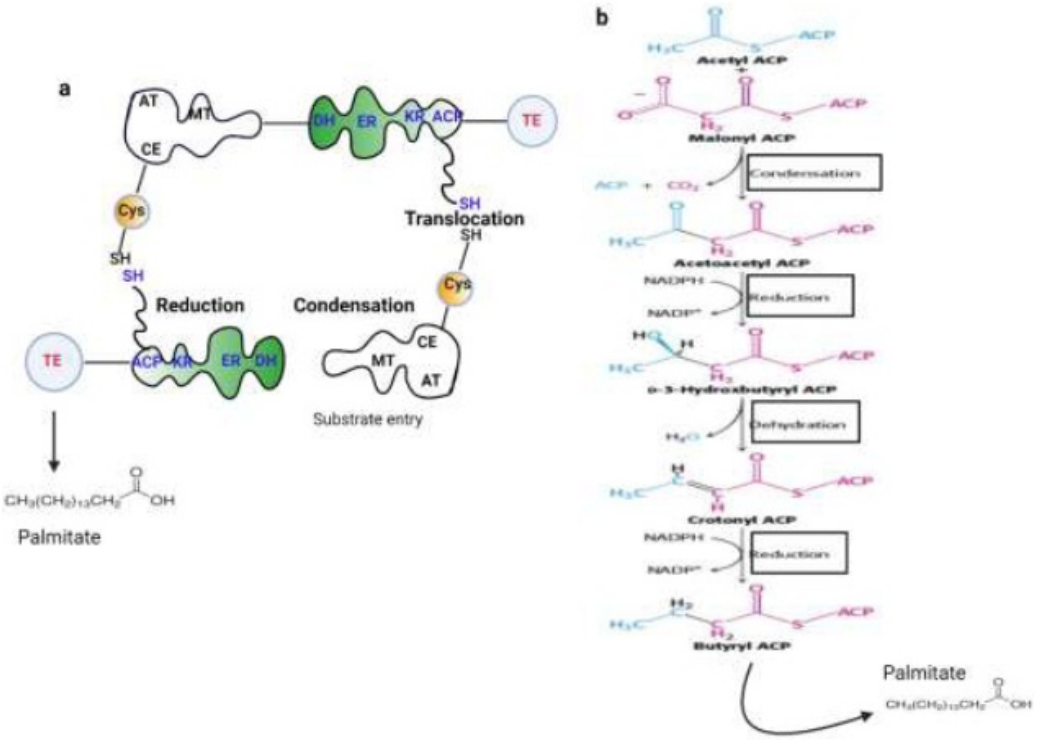



**Fig. (9.9). Schematic representation of fatty acid synthase.a)** Structure and domains of fatty acid synthase dimer. **B)** Fatty acid synthase system and its associated reactions (condensation, reduction, dehydration, and reduction) for palmitate synthesis. AT, acetyl transferase; CE, condensing enzyme; MT, malonyl transferase.

In the fatty acid synthase system, the acetyl group of acetyl CoA is transferred first to a serine residue in the active site of acetyl transferase and then to the sulfur atom of a cysteine residue in the active site of the condensing enzyme on one chain of the dimeric enzyme. Similarly, the malonyl group is transferred from malonyl CoA first to a serine residue in the active site of malonyl transferase and then to the sulfur atom of the phosphopantetheinyl group of the acyl carrier protein on the other chain in the dimer. Domain 1 of each chain of this dimer interacts with domains 2 and 3 of the other chain [41]. Next, the elongation starts with the joining of the acetyl unit on the condensing enzyme to a two-carbon part of the malonyl unit on the acyl carrier protein, resulting in the formation of an acetoacetyl-S-phosphopantetheinyl unit and the release of the $CO_2$ molecule. Further, the acetoacetyl group is delivered to three active sites in domain 2 of the opposite chain to reduce it to a butyryl unit (Fig. **9.9**), which is a saturated C4 unit. Butyril migrates from the phosphopantetheinyl sulfur atom on acyl carrier protein to the cysteine sulfur atom on the condensing enzyme, where it attaches to two-carbon part of the malonyl unit on the acyl carrier protein to form six-carbon

unit. This latter undergoes a reduction reaction. Fatty acid synthase performs five more rounds of condensation and reduction to produce a palmitoyl (C16) chain on the condensing enzyme. Palmitoyl is then hydrolyzed to palmitate through the thioesterase on domain 3 of the opposite chain. The reaction of palmitate synthesis by FAS requires 8 molecules of acetyl-CoA, 14 NADPH, and 7 ATPs and occurs in the cytosol, however acetyl-CoA is formed from pyruvate in the mitochondria (see chapter 8, section 6.3.2.1). As mitochondria are not permeable to acetyl-CoA, citrate is formed in the mitochondrial matrix by the condensation of acetyl-CoA with oxaloacetate. When its mitochondrial concentration is high, citrate is transported to the cytosol and cleaved by ATP citrate lyase (ACLY) to form acetyl-CoA and oxaloacetate (Fig. **9.10**). Similarly, mitochondria are impermeable to oxaloacetate, thus cytosol oxaloacetate is reduced to malate by malate dehydrogenase and NADH. Next, malate is decarboxylated by the malic enzyme to form pyruvate, which is able to enter the mitochondria, where it produces oxaloacetate via pyruvate carboxylase. For the 8 molecules of acetyl-CoA needed, there is a transfer of 8 molecules of mitochondrial NADPH to the cytosol. The rest of six NADPH required are originated from the pentose phosphate pathway (see chapter 8, section 8.3.2.9).

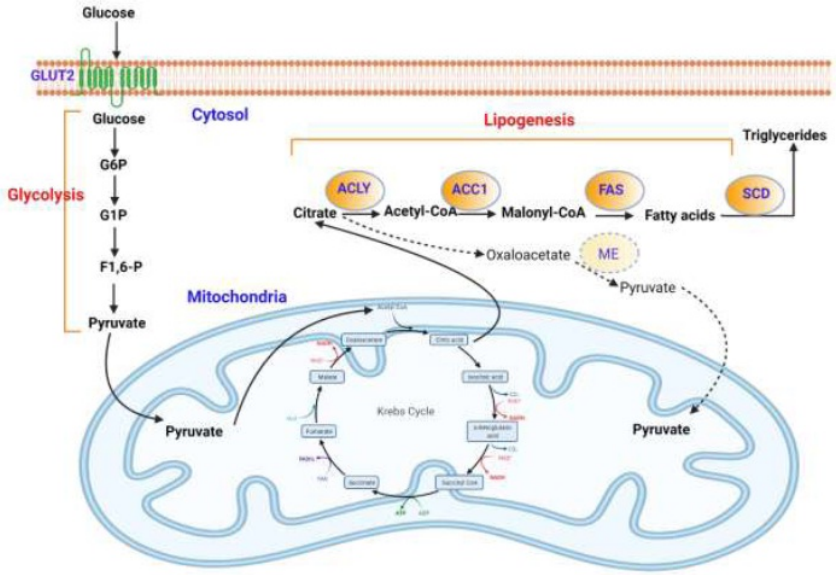



**Fig. (9.10). Schematic representation of hepatic lipogenesis.** After the consumption of carbohydrates, a portion of the circulating glucose is taken by hepatocytes through GLUT2, and then through glycolysis in the cytosol, glucose is converted to pyruvate, which is transported into mitochondria for further oxidation in the Kreb's cycle. Citrate, an intermediate of the Kreb's cycle, is exported into cytosol and used as a substrate for *de novo* lipogenesis. ACc1, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; FAS, fatty acid synthase; ME, malic enzyme; SCD, stearoyl-CoA desaturase.

The synthesized fatty acid can undergo elongation and/or desaturation. Elongation can occurs in the endoplasmic reticulum or in the mitochondria. Desaturation occurs in the endoplasmic reticulum. The elongation reaction is catalyzed by fatty acid elongase and it is similar to fatty acid synthesis, in which the source of the two-carbon unit is malonyl-CoA and the reducing power is provided by NADPH. Double bonds may be added via desaturating enzymes, such as Δ-5 desaturase, Δ-6 desaturase, and Δ-9 desaturase, in the endoplasmic reticulum. For instance, stearic acid (C18:0) is desaturated by Δ-9 desaturase to form oleic acid (C18:1 ω9). As described is section 9.1.2, human lacks the enzyme to desaturate beyond the ninth carbon of fatty acids, and therefore linoleic, and linolenic acids are dietary essentials.

## 9.5. Fatty Acid Degradation (Lipolysis)

White adipose tissue triglyceride is the major energy reserve in eukaryotes. During times of energy depletion, white adipose tissue undergoes a great rate of lipolysis, which can be defined as the hydrolysis of triglycerides to generate fatty acids (FAs) and glycerol that are released into the vasculature for use by other organs as energy substrates. Lipolysis proceeds in an orderly and regulated manner. First triglycerides are hydrolyzed by several lipases, including hormone-sensitive lipase [42, 43], adipose triglyceride lipase (or desnutrin) [44, 45], triacylglycerol hydrolases (TGH), also known as carboxylesterases [46, 47], adiponutrin [48], GS2 and GS2-like lipases [49]. The second step of lipolysis involves the hydrolysis of diacylglycerols to yield monoacylglycerols and a nonesterified fatty acid. This reaction occurs at a rate 10- to 30-folds higher than the hydrolysis of triglycerides, which is the initiating and rate-limiting step in lipolysis [50]. To date, Hormone-sensitive lipase is the only diacylglycerol lipase identified in adipocytes so far. Studies in rodent models deficient in HSL have confirmed the importance of HSL for the breakdown of diacylglycerols [51]. The last step in the lipolysis process is the hydrolysis of monoacylglycerols. The monoacylglycerol lipase (MGL) is a 33 kDa hydrolase discovered in 1975 [52]. MGL hydrolyzes the 1 and 2-ester bonds of monoacylglycerol at equal rates [53].

Several other hormones were found to play key roles in lipid hydrolysis. For instance, perilipin A and B isoforms are lipid droplet-associated proteins [54], and much evidence supports a complex role for them in regulating both basal and stimulated lipolysis [55 - 58]. As maximal rates of lipolysis require the removal of fatty acids from the adipocyte in order to prevent accumulation of reaction products and feedback inhibition of lipases, cytosolic lipid-binding proteins, such as FABP, ALBP, and aP2, play crucial roles in lipolysis [59]. Cohen and colleagues have shown a key role for caveolin-1 in the modulation of lipolysis and

lipid droplet formation [60]. Recently, several other proteins were shown to be involved in lipolysis, such as Comparative Gene Identification 58 (CGI-58), AQP7, and lipotransin. CGI-58 has been found to be the cause of the Chanarin-Dorfman syndrome (CDS), a rare autosomal recessive disease characterized by excessive accumulation of triacylglycerols in many organs [61, 62]. Rodents deficient in aquaporin 7 have impaired glycerol release in response to fasting and develop age-associated obesity caused by an induction of glycerol kinase and increased storage of triacylglycerols [63]. Lipotransin is an HSL-interacting protein that functions as a docking protein mediating the hormonally induced translocation of HSL from the cytoplasm to the lipid droplet [64].

## 9.6. Fatty Acid Oxidation

As described in previous chapters, fatty acids are important nutrients, and their storage as triglycerides in adipose tissue allows humans to tolerate extended periods of starvation or fasting and other metabolically challenging conditions such as f illness and exercise. The major pathway for the degradation of long-chain fatty acids is mitochondrial fatty acid β-oxidation, which was discovered by the German chemist Georg Franz Knoop in 1905. Fatty acid oxidation, composed of approximately of 20 different proteins, not only fuels the Krebs cycle and oxidative phosphorylation, but also stimulates hepatic synthesis of the ketone bodies. The uptake of fatty acids by the cells is mediated by simple diffusion as well as by specific proteins such as CD36 and plasma membrane fatty acid binding protein ($FABP_{pm}$, also known as GOT2). Once in the cytosol, fatty acids are converted to acyl-CoA esters by acyl-CoA synthetases, and then they can be directed into beta oxidation or lipid synthesis [65]. As the mitochondria membrane is impermeable to acyl-CoA, the carnitine shuttle is needed for import into the mitochondria. The carnitine palmitoyltransferase 1 (CPT1), an integral outer-mitochondrial-membrane protein, catalyzes the transesterification of the acyl-CoA to acylcarnitine [66]. Acylcarnitines is then transported into the mitochondria by carnitine acylcarnitine translocase (CACT) in exchange for free carnitine molecule [67]. Inside the mitochondria, the peripheral inner-mitochondrial-membrane protein CPT2 reconverts the acylcarnitine into an acyl-CoA (Fig. **9.11**). Most carnitine is of dietary origin and is transported across the plasma membrane by the organic cation transporter OCTN2 (SLC22A5) [68]. Next, acyl-CoA enters the mitochondrial β-oxidation, which consists of four enzymatic cyclic process. The cycle begins with dehydrogenation of the acyl-CoA to trans-2-enoyl-CoA by an acyl-CoA dehydrogenase. This step is followed by hydration catalyzed by an enoyl-CoA hydratase, generating (S)-3-hydroxyacl-CoA, which is subsequently dehydrogenated to 3-ketoacyl-CoA in a reaction performed by (S)-3-hydroxyacyl-CoA dehydrogenase. In the last step, a thiolase

cleaves the 3-ketoacyl-CoA into a two-carbon chain–shortened acetyl-CoA and acyl-CoA (Fig. **9.11**), which will be further used in Krebs cycle and ketogenesis, respectively. During these cycles, FAD and NAD are reduced to $FADH_2$ and NADH, respectively, which are used in the respiratory chain.

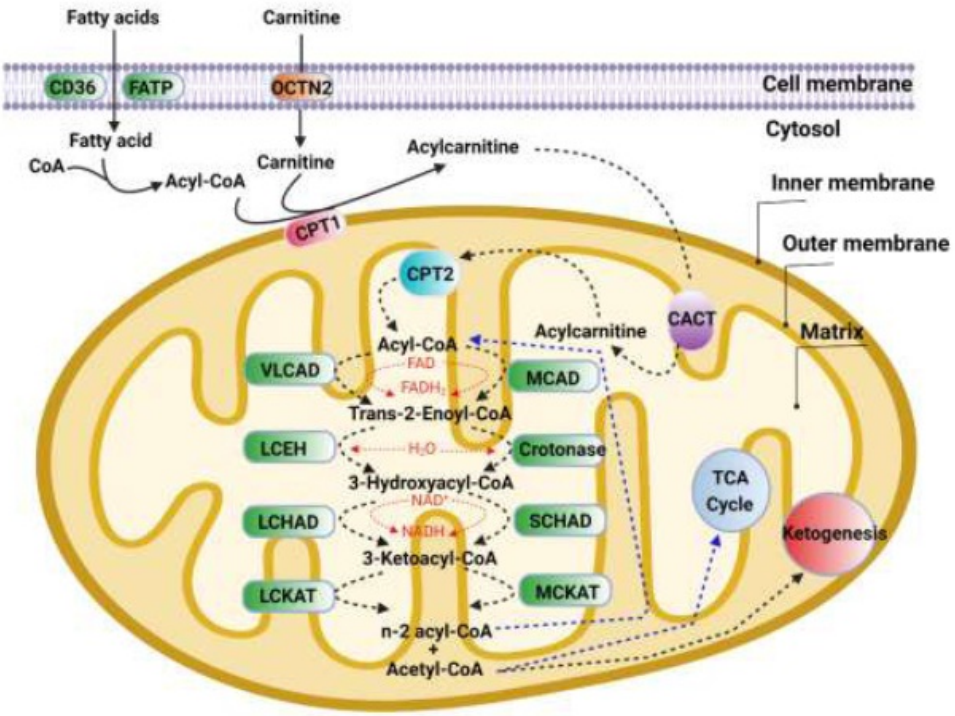



**Fig. (9.11). Schematic representation of mitochondrial fatty acid β-oxidation.** Fatty acids enter the cell by diffusion or via CD36 and FATP. In the cytosol, Fatty acids are converted to acylcarnitine by CPT1, which is translocated into the mitochondria via carnitine acylcarnitine translocase. In the mitochondria, acylcarnitine is converted back to acyl-CoA by CPT2. Mitochondrial acyl-CoA undergoes β-oxidation, which involves several enzymatic reactions, to generate n-2 acyl-CoA and acetyl-CoA. CD36, a cluster of differentiation 36; CPT, carnitine palmitoyltransferase; FAD, Flavin adenine dinucleotide; $FADH_2$, reduced Flavin adenine dinucleotide; FATP, fatty acid transport protein; LCEH, long-chain enoyl-CoA hydratase; LCHAD, long-chain (S)-3-hydroxyacyl-CoA dehydrogenase; LCKAT, long-chain 3-ketoacyl-CoA thiolase; MCAD, medium-chain acyl-CoA dehydrogenase; MCKAT, medium-chain 3-ketoacyl-CoA thiolase; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; SCHAD, short-chain (S)-3-hydroxyacyl-CoA dehydrogenase; VLCAD, very long chain acyl-CoA dehydrogenase.

## 9.7. Ketogenesis and Ketone Body Metabolism

Ketone bodies are a vital alternative metabolic fuel source during intracellular energy depletion [69]. In humans, ketone body metabolism has been leveraged to fuel the brain during episodic periods of nutrient deprivation. As described above, mammalian ketogenesis occurs predominantly in the liver mitochondria from β-

oxidation-derived acetyl-CoA, and the ketone bodies are transported to extrahepatic tissues for terminal oxidation [70]. Blood total ketone body concentration is approximately 100–250 μM in healthy adult humans, rises to ~1 mM after prolonged exercise or 24h of fasting, and can accumulate to as high as 20 mM in pathological states like diabetic ketoacidosis [71, 72]. Interestingly, the human liver produces approximately 300g of ketone bodies per day [73].

Ketogenesis starts when the mitochondrial β-oxidation-derived acetyl-CoA exceeds citrate synthase activity and/or oxaloacetate availability for condensation to form citrate. Hence, it is classically viewed as a spillover pathway. Acetyl-CoA is condensed with acetoacetyl-CoA via the mitochondrial isoform 3-hydroxymethylglutaryl-CoA synthase (HMGCS2) to produce hydroxymethylglutaryl-CoA (HMG-CoA), which in turn is cleaved into acetyl-CoA and acetoacetate through HMG-CoA lyase (HMGCL) (Fig. **9.12**). Acetoacetate is reduced to D-β-hydroxybutyrate (D-βOHB) by phosphatidylcholine-dependent mitochondrial d-βOHB dehydrogenase (BDH1) in a $NAD^+$/NADH-coupled reaction [74]. Acetoacetate can also be spontaneously converted to acetone through a non-enzymatic decarboxylation.

Although the mechanisms by which acetoacetate and beta-hydroxybutyrate are transported across the mitochondrial inner membrane are not well defined, these ketone bodies are secreted from the hepatic cells via monocarboxylate transporters (MCT1/2), also known as solute carrier 16A family members 1 and 7 [75]. Once reach extrahepatic tissues, ketone bodies can be:

1. Catabolized in the mitochondria to produce acetyl-CoA that can be used in the Krebs cycle. For instance, D-βOHB is converted back to acetoacetate, which is activated to acetoacetyl-CoA through the exchange of a CoA-moiety from succinyl-CoA in a reaction catalyzed by the CoA transferase, succinyl-CoA:--oxoacid-CoA transferase (SCOT, encoded by OXCT1 gene). Acetoacetyl-CoA is then converted to acetyl-CoA, via mitochondrial thiolases (ACAA2, ACAT1, HADHA, or HADHB), which enter Krebs cycle.
2. Used in the lipogenesis or sterol synthesis pathways. In fact, cytosolic acetoacetyl-CoA can be either directed by cytosolic HMGCS1 toward sterol biosynthesis, or cleaved by the cytoplasmic thiolases (ACAAs) to acetyl-CoA, which is carboxylated to malonyl-CoA via acetyl-coA carboxylase. Malonyl-CoA enters the *de novo* lipogenesis pathway.
3. Excreted in the urine

Acetoacetate and beta-hydroxybutyrate are the two ketone bodies used by the body for energy, and they are avidly oxidized in heart, brain, and skeletal muscle [76].

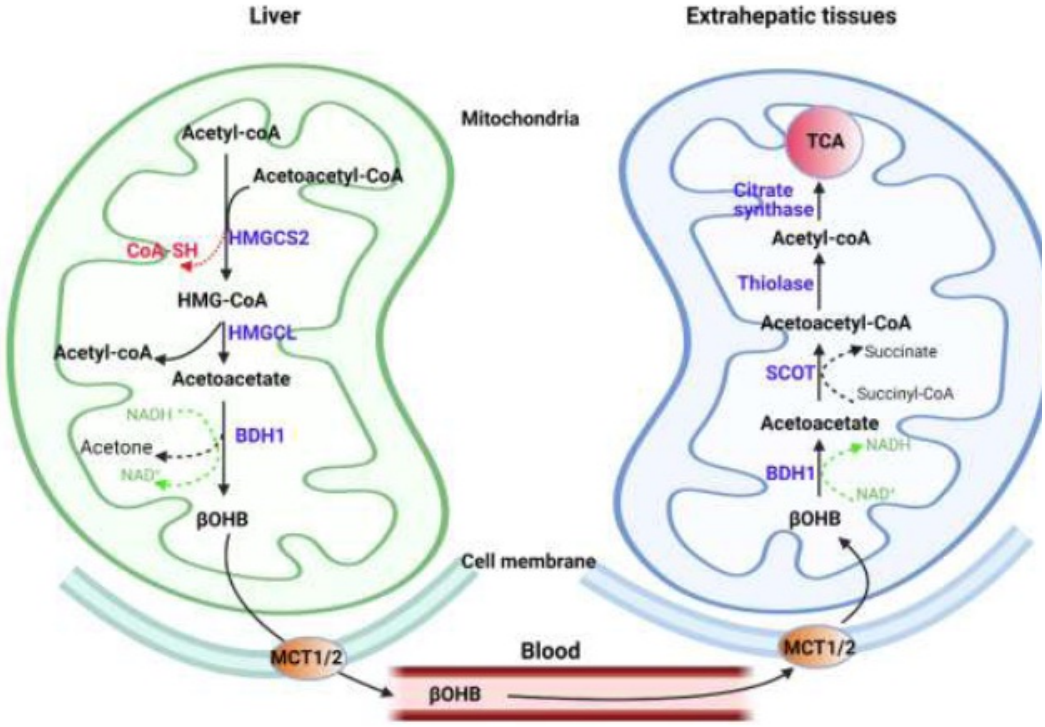



**Fig. (9.12). Schematic representation of mitochondrial ketogenesis.** Ketogenesis within hepatic mitochondria is the primary source of circulating ketone bodies. Acetyl-CoA is condensed with acetoacetyl-CoA via 3-hydroxymethylglutaryl-CoA synthase to produce hydroxymethylglutaryl-CoA, which in turn is cleaved into acetyl-CoA and acetoacetate through HMG-CoA lyase. Acetoacetate is reduced to D--hydroxybutyrate (βOHB) by phosphatidylcholine-dependent mitochondrial d-βOHB dehydrogenase (BDH1). βOHB is released through MCT1/2 and transported to extrahepatic tissues, where it is converted to acetoacetate, acetoacetyl-coA, and acetyl-coA via BDH1, SCOT, and thiolases, respectively. Acetyl-CoA can be used in the Krebs cycle to generate ATP.

## CONCLUSION

Lipid metabolism involves the synthesis and the degradation of fat to satisfy the metabolic needs of the cell. These lipids play critical structural and functional roles. They are in the constant dynamic between oxidation and storage. Dysregulation of this equilibrium alters the cellular homeostasis and can lead to metabolic disorders.

## NOTES

[1] Chyme is semi-fluid pulp formed in the stomach made of partly digested food and the secretions of the GI tract.

[2] Emulsification is a process in which large lipid globules are broken down into several small lipid globules

[3] Micelles are spherical form of lipids in aqueous solutions.

[4] Lipogenesis is the metabolic formation of fat (conversion of acetyl-CoA to triglycerides: de novo fatty acid synthesis).

## REFERENCES

[1] Zheng L, Fleith M, Giuffrida F, O'Neill BV, Schneider N. Dietary Polar Lipids and Cognitive Development: A Narrative Review. Adv Nutr 2019; 10(6): 1163-76.
[http://dx.doi.org/10.1093/advances/nmz051] [PMID: 31147721]

[2] Lagrost L. Differential effects of cis and trans fatty acid isomers, oleic and elaidic acids, on the cholesteryl ester transfer protein activity. Biochim Biophys Acta Lipids Lipid Metab 1992; 1124(2): 159-62.
[http://dx.doi.org/10.1016/0005-2760(92)90092-A] [PMID: 1543738]

[3] Rajaram S. Health benefits of plant-derived α-linolenic acid. Am J Clin Nutr 2014; 100 (Suppl. 1): 443S-8S.
[http://dx.doi.org/10.3945/ajcn.113.071514] [PMID: 24898228]

[4] Di Pasquale MG. The essentials of essential fatty acids. J Diet Suppl 2009; 6(2): 143-61.
[http://dx.doi.org/10.1080/19390210902861841] [PMID: 22435414]

[5] Ayerza R Jr, Coates W. Effect of dietary alpha-linolenic fatty acid derived from chia when fed as ground seed, whole seed and oil on lipid content and fatty acid composition of rat plasma. Ann Nutr Metab 2007; 51(1): 27-34.
[http://dx.doi.org/10.1159/000100818] [PMID: 17356263]

[6] Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder PC. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. Prog Lipid Res 2016; 64: 30-56.
[http://dx.doi.org/10.1016/j.plipres.2016.07.002] [PMID: 27496755]

[7] Burdge GC, Calder PC. Dietary α-linolenic acid and health-related outcomes: a metabolic perspective. Nutr Res Rev 2006; 19(1): 26-52.
[http://dx.doi.org/10.1079/NRR2005113] [PMID: 19079874]

[8] Lagace TA, Ridgway ND. The role of phospholipids in the biological activity and structure of the endoplasmic reticulum. Biochim Biophys Acta Mol Cell Res 2013; 1833(11): 2499-510.
[http://dx.doi.org/10.1016/j.bbamcr.2013.05.018] [PMID: 23711956]

[9] Meng Y, Heybrock S, Neculai D, Saftig P. Cholesterol Handling in Lysosomes and Beyond. Trends Cell Biol 2020; 30(6): 452-66.
[http://dx.doi.org/10.1016/j.tcb.2020.02.007] [PMID: 32413315]

[10] Gimpl G, Wiegand V, Burger K, Fahrenholz F. Cholesterol and steroid hormones: modulators of oxytocin receptor function. Prog Brain Res 2002; 139: 43-55.
[http://dx.doi.org/10.1016/S0079-6123(02)39006-X] [PMID: 12436925]

[11] Hume R, Boyd GS. Cholesterol metabolism and steroid-hormone production. Biochem Soc Trans 1978; 6(5): 893-8.
[http://dx.doi.org/10.1042/bst0060893] [PMID: 744338]

[12] Sato R. Recent advances in regulating cholesterol and bile acid metabolism. Biosci Biotechnol Biochem 2020; 84(11): 2185-92.

[http://dx.doi.org/10.1080/09168451.2020.1793658] [PMID: 32657638]

[13] Javitt NB. Cholesterol, hydroxycholesterols, and bile acids. Biochem Biophys Res Commun 2002; 292(5): 1147-53.
[http://dx.doi.org/10.1006/bbrc.2001.2013] [PMID: 11969205]

[14] Hamosh M, Scow RO. Lingual lipase and its role in the digestion of dietary lipid. J Clin Invest 1973; 52(1): 88-95.
[http://dx.doi.org/10.1172/JCI107177] [PMID: 4682389]

[15] Mu H, Høy CE. The digestion of dietary triacylglycerols. Prog Lipid Res 2004; 43(2): 105-33.
[http://dx.doi.org/10.1016/S0163-7827(03)00050-X] [PMID: 14654090]

[16] Iqbal J, Hussain MM. Intestinal lipid absorption. Am J Physiol Endocrinol Metab 2009; 296(6): E1183-94.
[http://dx.doi.org/10.1152/ajpendo.90899.2008] [PMID: 19158321]

[17] Abumrad N, Harmon C, Ibrahimi A. Membrane transport of long-chain fatty acids: evidence for a facilitated process. J Lipid Res 1998; 39(12): 2309-18.
[http://dx.doi.org/10.1016/S0022-2275(20)33310-1] [PMID: 9831619]

[18] Storch J, Thumser AE. Tissue-specific functions in the fatty acid-binding protein family. J Biol Chem 2010; 285(43): 32679-83.
[http://dx.doi.org/10.1074/jbc.R110.135210] [PMID: 20716527]

[19] Pan X, Hussain MM. Gut triglyceride production. Biochim Biophys Acta Mol Cell Biol Lipids 2012; 1821(5): 727-35.
[http://dx.doi.org/10.1016/j.bbalip.2011.09.013] [PMID: 21989069]

[20] Demignot S, Beilstein F, Morel E. Triglyceride-rich lipoproteins and cytosolic lipid droplets in enterocytes: Key players in intestinal physiology and metabolic disorders. Biochimie 2014; 96: 48-55.
[http://dx.doi.org/10.1016/j.biochi.2013.07.009] [PMID: 23871915]

[21] Martin S, Parton RG. Lipid droplets: a unified view of a dynamic organelle. Nat Rev Mol Cell Biol 2006; 7(5): 373-8.
[http://dx.doi.org/10.1038/nrm1912] [PMID: 16550215]

[22] Ohsaki Y, Cheng J, Suzuki M, Shinohara Y, Fujita A, Fujimoto T. Biogenesis of cytoplasmic lipid droplets: From the lipid ester globule in the membrane to the visible structure. Biochim Biophys Acta Mol Cell Biol Lipids 2009; 1791(6): 399-407.
[http://dx.doi.org/10.1016/j.bbalip.2008.10.002] [PMID: 18996222]

[23] Mahmood Hussain M, Kancha RK, Zhou Z, Luchoomun J, Zu H, Bakillah A. Chylomicron assembly and catabolism: role of apolipoproteins and receptors. Biochim Biophys Acta Lipids Lipid Metab 1996; 1300(3): 151-70.
[http://dx.doi.org/10.1016/0005-2760(96)00041-0] [PMID: 8679680]

[24] Hussain MM, Shi J, Dreizen P. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. J Lipid Res 2003; 44(1): 22-32.
[http://dx.doi.org/10.1194/jlr.R200014-JLR200] [PMID: 12518019]

[25] Jiang ZG, Liu Y, Hussain MM, Atkinson D, McKnight CJ. Reconstituting initial events during the assembly of apolipoprotein B-containing lipoproteins in a cell-free system. J Mol Biol 2008; 383(5): 1181-94.
[http://dx.doi.org/10.1016/j.jmb.2008.09.006] [PMID: 18804479]

[26] Luchoomun J, Hussain MM. Assembly and secretion of chylomicrons by differentiated Caco-2 cells. Nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly. J Biol Chem 1999; 274(28): 19565-72.
[http://dx.doi.org/10.1074/jbc.274.28.19565] [PMID: 10391890]

[27] Kumar NS, Mansbach CM II. Prechylomicron transport vesicle: isolation and partial characterization. Am J Physiol 1999; 276(2): G378-86.

[PMID: 9950811]

[28] Siddiqi SA, Mahan J, Siddiqi S, Gorelick FS, Mansbach CM II. Vesicle-associated membrane protein 7 is expressed in intestinal ER. J Cell Sci 2006; 119(5): 943-50.
[http://dx.doi.org/10.1242/jcs.02803] [PMID: 16495485]

[29] Siddiqi S, Saleem U, Abumrad NA, *et al.* A novel multiprotein complex is required to generate the prechylomicron transport vesicle from intestinal ER. J Lipid Res 2010; 51(7): 1918-28.
[http://dx.doi.org/10.1194/jlr.M005611] [PMID: 20237389]

[30] Tiwari S, Siddiqi SA. Intracellular trafficking and secretion of VLDL. Arterioscler Thromb Vasc Biol 2012; 32(5): 1079-86.
[http://dx.doi.org/10.1161/ATVBAHA.111.241471] [PMID: 22517366]

[31] Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell 2015; 161(1): 161-72.
[http://dx.doi.org/10.1016/j.cell.2015.01.036] [PMID: 25815993]

[32] Zhang L, Reue K, Fong LG, Young SG, Tontonoz P. Feedback regulation of cholesterol uptake by the LXR-IDOL-LDLR axis. Arterioscler Thromb Vasc Biol 2012; 32(11): 2541-6.
[http://dx.doi.org/10.1161/ATVBAHA.112.250571] [PMID: 22936343]

[33] Trigatti BL Sr. SR-B1 and PDZK1. Curr Opin Lipidol 2017; 28(2): 201-8.
[http://dx.doi.org/10.1097/MOL.0000000000000396] [PMID: 28134663]

[34] Svensson RU, Parker SJ, Eichner LJ, *et al.* Inhibition of acetyl-CoA carboxylase suppresses fatty acid synthesis and tumor growth of non-small-cell lung cancer in preclinical models. Nat Med 2016; 22(10): 1108-19.
[http://dx.doi.org/10.1038/nm.4181] [PMID: 27643638]

[35] Hunkeler M, Hagmann A, Stuttfeld E, *et al.* Structural basis for regulation of human acetyl-CoA carboxylase. Nature 2018; 558(7710): 470-4.
[http://dx.doi.org/10.1038/s41586-018-0201-4] [PMID: 29899443]

[36] Bianchi A, Evans JL, Iverson AJ, Nordlund AC, Watts TD, Witters LA. Identification of an isozymic form of acetyl-CoA carboxylase. J Biol Chem 1990; 265(3): 1502-9.
[http://dx.doi.org/10.1016/S0021-9258(19)40045-8] [PMID: 1967254]

[37] Abu-Elheiga L, Brinkley WR, Zhong L, Chirala SS, Woldegiorgis G, Wakil SJ. The subcellular localization of acetyl-CoA carboxylase 2. Proc Natl Acad Sci USA 2000; 97(4): 1444-9.
[http://dx.doi.org/10.1073/pnas.97.4.1444] [PMID: 10677481]

[38] Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer 2007; 7(10): 763-77.
[http://dx.doi.org/10.1038/nrc2222] [PMID: 17882277]

[39] Chakravarty B, Gu Z, Chirala SS, Wakil SJ, Quiocho FA. Human fatty acid synthase: Structure and substrate selectivity of the thioesterase domain. Proc Natl Acad Sci USA 2004; 101(44): 15567-72.
[http://dx.doi.org/10.1073/pnas.0406901101] [PMID: 15507492]

[40] Bueno MJ, Jimenez-Renard V, Samino S, *et al.* Essentiality of fatty acid synthase in the 2D to anchorage-independent growth transition in transforming cells. Nat Commun 2019; 10(1): 5011.
[http://dx.doi.org/10.1038/s41467-019-13028-1] [PMID: 31676791]

[41] 2002.

[42] Holm C. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Biochem Soc Trans 2003; 31(6): 1120-4.
[http://dx.doi.org/10.1042/bst0311120] [PMID: 14641008]

[43] Osuga J, Ishibashi S, Oka T, *et al.* Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. Proc Natl Acad Sci USA 2000; 97(2): 787-92.
[http://dx.doi.org/10.1073/pnas.97.2.787] [PMID: 10639158]

[44] Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, Sul HS. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J Biol Chem 2004; 279(45): 47066-75.
[http://dx.doi.org/10.1074/jbc.M403855200] [PMID: 15337759]

[45] Zimmermann R, Strauss JG, Haemmerle G, *et al.* Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 2004; 306(5700): 1383-6.
[http://dx.doi.org/10.1126/science.1100747] [PMID: 15550674]

[46] Soni KG, Lehner R, Metalnikov P, *et al.* Carboxylesterase 3 (EC 3.1.1.1) is a major adipocyte lipase. J Biol Chem 2004; 279(39): 40683-9.
[http://dx.doi.org/10.1074/jbc.M400541200] [PMID: 15220344]

[47] Lehner R, Vance DE. Cloning and expression of a cDNA encoding a hepatic microsomal lipase that mobilizes stored triacylglycerol. Biochem J 1999; 343(1): 1-10.
[http://dx.doi.org/10.1042/bj3430001] [PMID: 10493905]

[48] Lake AC, Sun Y, Li JL, *et al.* Expression, regulation, and triglyceride hydrolase activity of Adiponutrin family members. J Lipid Res 2005; 46(11): 2477-87.
[http://dx.doi.org/10.1194/jlr.M500290-JLR200] [PMID: 16150821]

[49] Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 2004; 279(47): 48968-75.
[http://dx.doi.org/10.1074/jbc.M407841200] [PMID: 15364929]

[50] Giudicelli H, Pastre N, Boyer J. Lipolytic activity of adipose tissue. Biochim Biophys Acta Lipids Lipid Metab 1974; 348(2): 221-31.
[http://dx.doi.org/10.1016/0005-2760(74)90233-1] [PMID: 4847556]

[51] Haemmerle G, Zimmermann R, Hayn M, *et al.* Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. J Biol Chem 2002; 277(7): 4806-15.
[http://dx.doi.org/10.1074/jbc.M110355200] [PMID: 11717312]

[52] Tornqvist H, Belfrage P. Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue. J Biol Chem 1976; 251(3): 813-9.
[http://dx.doi.org/10.1016/S0021-9258(17)33857-7] [PMID: 1249056]

[53] Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 1997; 272(43): 27218-23.
[http://dx.doi.org/10.1074/jbc.272.43.27218] [PMID: 9341166]

[54] Greenberg AS, Egan JJ, Wek SA, Moos MC Jr, Londos C, Kimmel AR. Isolation of cDNAs for perilipins A and B: sequence and expression of lipid droplet-associated proteins of adipocytes. Proc Natl Acad Sci USA 1993; 90(24): 12035-9.
[http://dx.doi.org/10.1073/pnas.90.24.12035] [PMID: 7505452]

[55] Tansey JT, Sztalryd C, Gruia-Gray J, *et al.* Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. Proc Natl Acad Sci USA 2001; 98(11): 6494-9.
[http://dx.doi.org/10.1073/pnas.101042998] [PMID: 11371650]

[56] Tansey JT, Huml AM, Vogt R, *et al.* Functional studies on native and mutated forms of perilipins. A role in protein kinase A-mediated lipolysis of triacylglycerols. J Biol Chem 2003; 278(10): 8401-6.
[http://dx.doi.org/10.1074/jbc.M211005200] [PMID: 12477720]

[57] Sztalryd C, Xu G, Dorward H, *et al.* Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. J Cell Biol 2003; 161(6): 1093-103.
[http://dx.doi.org/10.1083/jcb.200210169] [PMID: 12810697]

[58] Miyoshi H, Souza SC, Zhang HH, *et al.* Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms. J Biol Chem 2006; 281(23): 15837-44.
[http://dx.doi.org/10.1074/jbc.M601097200] [PMID: 16595669]

[59] Coe NR, Simpson MA, Bernlohr DA. Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. J Lipid Res 1999; 40(5): 967-72.
[http://dx.doi.org/10.1016/S0022-2275(20)32133-7] [PMID: 10224167]

[60] Cohen AW, Razani B, Schubert W, *et al.* Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. Diabetes 2004; 53(5): 1261-70.
[http://dx.doi.org/10.2337/diabetes.53.5.1261] [PMID: 15111495]

[61] Lefèvre C, Jobard F, Caux F, *et al.* Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. Am J Hum Genet 2001; 69(5): 1002-12.
[http://dx.doi.org/10.1086/324121] [PMID: 11590543]

[62] Lass A, Zimmermann R, Haemmerle G, *et al.* Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. Cell Metab 2006; 3(5): 309-19.
[http://dx.doi.org/10.1016/j.cmet.2006.03.005] [PMID: 16679289]

[63] Maeda N, Funahashi T, Hibuse T, *et al.* Adaptation to fasting by glycerol transport through aquaporin 7 in adipose tissue. Proc Natl Acad Sci USA 2004; 101(51): 17801-6.
[http://dx.doi.org/10.1073/pnas.0406230101] [PMID: 15591341]

[64] Syu LJ, Saltiel AR. Lipotransin. Mol Cell 1999; 4(1): 109-15.
[http://dx.doi.org/10.1016/S1097-2765(00)80192-6] [PMID: 10445032]

[65] Grevengoed TJ, Klett EL, Coleman RA. Acyl-CoA metabolism and partitioning. Annu Rev Nutr 2014; 34(1): 1-30.
[http://dx.doi.org/10.1146/annurev-nutr-071813-105541] [PMID: 24819326]

[66] Ma Y, Wang W, Devarakonda T, *et al.* Functional analysis of molecular and pharmacological modulators of mitochondrial fatty acid oxidation. Sci Rep 2020; 10(1): 1450.
[http://dx.doi.org/10.1038/s41598-020-58334-7] [PMID: 31996743]

[67] Ramon De Lucas J, Ramon De Lucas J, Indiveri C, *et al.* Functional characterization of residues within the carnitine/acylcarnitine translocase RX $_2$ PANAAXF distinct motif. Mol Membr Biol 2008; 25(2): 152-63.
[http://dx.doi.org/10.1080/09687680701697476] [PMID: 18307102]

[68] Vaz FM, Wanders RJA. Carnitine biosynthesis in mammals. Biochem J 2002; 361(3): 417-29.
[http://dx.doi.org/10.1042/bj3610417] [PMID: 11802770]

[69] Cahill GF Jr. Fuel metabolism in starvation. Annu Rev Nutr 2006; 26(1): 1-22.
[http://dx.doi.org/10.1146/annurev.nutr.26.061505.111258] [PMID: 16848698]

[70] McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem 1980; 49(1): 395-420.
[http://dx.doi.org/10.1146/annurev.bi.49.070180.002143] [PMID: 6157353]

[71] Johnson RH, Walton JL, Krebs HA, Williamson DH. Post-exercise ketosis. Lancet 1969; 294(7635): 1383-5.
[http://dx.doi.org/10.1016/S0140-6736(69)90931-3] [PMID: 4188274]

[72] Johnson RH, Walton JL, Krebs HA, Williamson DH. Metabolic fuels during and after severe exercise in athletes and non-athletes. Lancet 1969; 294(7618): 452-5.
[http://dx.doi.org/10.1016/S0140-6736(69)90164-0] [PMID: 4183902]

[73] Balasse EO, Féry F. Ketone body production and disposal: Effects of fasting, diabetes, and exercise. Diabetes Metab Rev 1989; 5(3): 247-70.
[http://dx.doi.org/10.1002/dmr.5610050304] [PMID: 2656155]

[74] Bock H, Fleischer S. Preparation of a homogeneous soluble D-beta-hydroxybutyrate apodehydrogenase from mitochondria. J Biol Chem 1975; 250(15): 5774-81.
[http://dx.doi.org/10.1016/S0021-9258(19)41121-6] [PMID: 1171099]

[75] Hugo SE, Cruz-Garcia L, Karanth S, Anderson RM, Stainier DYR, Schlegel A. A monocarboxylate transporter required for hepatocyte secretion of ketone bodies during fasting. Genes Dev 2012; 26(3): 282-93.
[http://dx.doi.org/10.1101/gad.180968.111] [PMID: 22302940]

[76] Reichard GA Jr, Owen OE, Haff AC, Paul P, Bortz WM. Ketone-body production and oxidation in fasting obese humans. J Clin Invest 1974; 53(2): 508-15.
[http://dx.doi.org/10.1172/JCI107584] [PMID: 11344564]

# CHAPTER 10

# Summary

We all need food and water! Eating and drinking are paramount to maintaining life via the provision of the necessary energy. For that end, consumed macronutrients undergo biochemical processes (metabolism) that include more than 8,700 reactions and 16,000 metabolites (http://www.genome.jp/kegg /pathway.html). In this first edition of *Nutritional Biochemistry: from the classroom to the Research Bench*, I tried to keep the textbook current with the most recent scientific advances, and at the same time, maintain a clear and readable style. As biochemical nutrition is an integrative science that encompasses knowledge, concepts, and methodology related to the chemical properties of macronutrients and their metabolic pathways and physiological functions, this book contains several chapters describing the body and the cell, defining macronutrients and food composition, and outlining the recent mechanisms involved in food and water intake, and their central and peripheral metabolic pathways. The whole objective of the book is summarized in Fig. (**10.1**). The target audience for this book is undergraduate and graduate students in the human and animal sciences, nutrition, dietetics, food sciences, veterinary and human medicine. Illustrating with figures, tables, and structural diagrams, my objective is to provide students and readers with sufficient biochemistry and pathways to understand the science of nutrition. I hope that this book is a great resource with great educational outcomes in value-added terms that help students to understand the intricacies of biochemical nutrition, grasp though concepts, and gain the necessary background to pursue higher-level nutrition classes, or simply acquire the necessary tool to design, conduct, interpret and/or discuss a biochemical nutrition experiment. I hope that you find the book useful and you enjoy reading it, however, if you have any comments for improvement, please do contact me, and I will make sure to include them in the next edition.

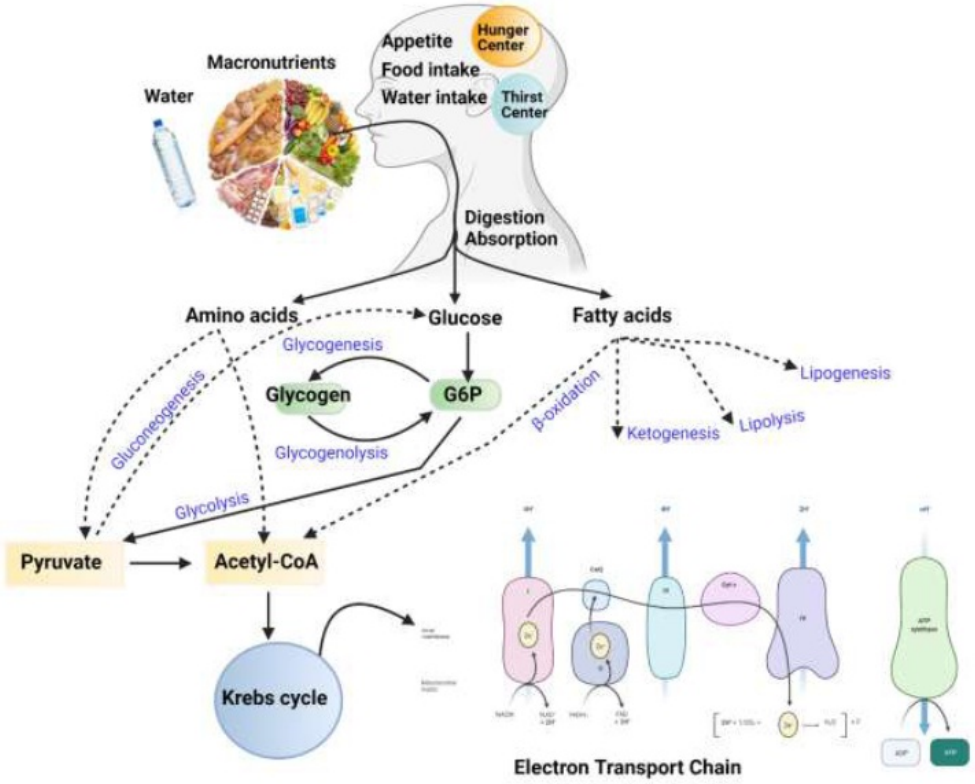



**Fig. (10.1). Schematic illustration and summary of the book's objectives.** The "*Nutritional Biochemistry: From the classroom to the Research Bench*" Book provides current knowledge related to energy homeostasis and integrates relevant principles in nutrition, physiology, biochemistry, and molecular signaling pathways that regulate water and food intake as well as their metabolism.

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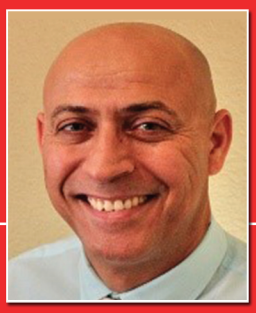

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Dr. Dridi is a Professor of avian endocrinology and molecular genetics at the Department of Poultry Science at the University of Arkansas. He earned his BS in math and science in 1990, his engineering degree in animal sciences from ESAM in 1995, his master of advanced studies (MAS) in agronomic sciences, molecular and cellular biology from National Polytechnic Institute of Lorraine (INPL), France, in 1998, his Ph.D. in poultry science, Molecular and Cellular Biology from INPL and National Institute of Agronomic Research (INRA) France, in 2001, and his accreditation to supervise research in structural biology, biochemistry and cell signaling from the University of Paris XI (France) in 2012. Dr. Dridi served as a quality inspector in the poultry industry from 1995-1997, and he joined several national and international labs as a postdoc/main investigator, such as UNC at Chapel Hill (USA), University of Kentucky (USA), University of West Virginia (USA), Catholic University of Leuven (Belgium), University of Bordeaux (France) and National Veterinary Institute of Nantes (France). He is a member of various scientific associations such as the American Physiological Society, American Association for the Advancement of Science, Poultry Science Association, and World Poultry Science Association. Dr. Dridi authored more than 260 papers (original research manuscripts, abstracts, and book chapters) and co-edited the avian physiology book edition 7. He plays an active role in mentoring undergraduate and graduate students and molding the next generation of poultry scientists. He served as a Reviewer and Editorial Member of several scientific journals and more than 50 advisory committees.