Advances in Health and Disease

Advances in Health and Disease

Volume 68

Lowell T. Duncan
Editor

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Contents

Preface

This book focuses on the latest developments in health and disease research.

Chapter 1 – Iron (Fe) plays a crucial role in living organisms' metabolism, anti-bacterial protection, oxygen transport, etc. Fe is important for life because of its wide availability – this element is one of the most popular ones on Earth. The Earth Core is also built from iron and nickel, and because of the very high pressure and temperature, both metals are present in a liquid form. A consequence of this fact is creating a magnetic field around the Earth as protection from the solar wind (ionized particles and g-radiation emitted by the sun). Humans also use iron for building and vehicle construction, engineering, electronics, and others.

The importance of Fe for living organisms, including the human, is related to the presence of Fe ions in selected proteins like ferritin, transferrins, and lactoferrin or lactotransferrin. These proteins play a crucial role in, for instance, the protection of the body from oxidative damage and the immune system of the body. Also, a metalloprotein like hemoglobin, built of prosthetic group Fe-containing, and proteins chains, is the species responsible for oxygen transport to tissues.

The importance of Fe is also related to the presence of redox-pair like $Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$ ($E = 0.77$ V), and this reaction is crucial, for instance, in oxygen releasing by hemoglobin in tissues: $4\,Fe^{2+} + O_2 \rightarrow 4\,Fe^{3+} + 2O^{2-}$. Human iron requirements are variable and depend on age, sex, and condition of the body (from 1 mg/day in men to 2 mg in women; however, during pregnancy and lactation, it should be approx. 3 mg/day). Fe deficit can cause an iron deficiency anemia. Measuring the Fe/ferritin level is used in determining anemia. High amounts of Fe(II) are toxic, and on the opposite, Fe(III-VI) compounds are not because they are not absorbed.

In this chapter, also Fe-S protein is discussed, which are proteins Fe-S clusters containing and connected via sulfur atoms.

Chapter 2 - Diabetes mellitus (DM) is a group of metabolic disorders in which various genetic and environmental factors result in the progressive

decline of beta-cell mass or function, characterized by elevated blood glucose levels, which is attributed to defects in insulin synthesis and its resistance. The molecular mechanism of pathogenesis in DM involves the deregulation of signaling pathways that regulate oxidative stress, endoplasmic reticulum (ER) stress, and inflammatory response. ER stress has been well-implicated as a crucial factor in the pathogenesis of DM, including inflammation, lipid accumulation, insulin biosynthesis, and β-cell apoptosis. Many signaling molecules and transcription factors, such as ATF6, XBP1, ATF4, NFκB, AP1, SREBP, and CHOP, have been associated with the ER stress response.

A better understanding of the molecular mechanisms disrupting ER homeostasis may offer new pharmacological targets for treating and preventing diabetes. This chapter elucidates the crucial aspects of ER stress, its manifestation in diabetes and complications, and obesity-mediated diabetes. The role of ER stress in exacerbating oxidative stress and inflammation during diabetes is also covered. The authors shed light on the crosstalk between ER stress mediators with oxidative and inflammatory stress mediators in the background of DM. The authors extensively summarize preclinical and clinical research evidence about the activation of nuclear receptors and cytoprotective mechanisms like Nrf2 during ER stress, and the role of therapeutic compounds in inhibiting ER stress in T2DM and its complications.

Chapter 3 - CD4+ T cells are a multi-faceted cellular component of the adaptive immune system. Upon activation, naïve CD4+ T cells differentiate into specific T helper (Th) cell subtypes depending on lineage-specific signature cytokines. The crucial function of these CD4+ T cells is to encounter and clear the invading pathogens by effectively coordinating a highly regulated adaptive immune response. However, CD4+ T cells are also known to drive inflammation and are associated with several autoimmune disorders.

In this chapter, the authors will discuss these CD4+ T cells and their characteristic T helper (Th) functions. Next, the authors will describe the role of cytokines, transcription factors, and metabolic pathways that determine the fate, plasticity, and heterogeneity of Th cells. Finally, the authors will illustrate the involvement of protective and hyper-inflammatory functions of Th subsets in various autoimmune diseases, cancer, and viral infections.

Chapter 4 - CD4 T helper (Th) cells are key T lymphocyte subsets that orchestrate the proper development of innate and adaptive immune responses. Th cells differentiate to several subtypes, such as Th1, Th2, Th17, and Th22 cells, which secrete distinct cytokines depending on the antigens encountered. Regulatory T (Treg) cells are another subgroup of CD4 T cells that act against

Th17. Sepsis is a life-threatening multiorgan dysfunction syndrome characterized by decreased Th cell numbers and impaired functions. Th cells play crucial roles in the induction and persistence of sepsis. Studies have reported that the dysregulation of CD4 T-cell subpopulations causes inflammatory reactions that promote sepsis progression.

Restoring the Th function and orchestrating the homeostasis of Th/Treg subpopulations are essential for managing sepsis and alleviating subsequent organ injury. Glutamine, arginine, n-3 polyunsaturated fatty acid, vitamin A, and vitamin D are specific nutrients with anti-inflammatory and immunomodulatory properties. Studies have demonstrated that additional supplementation of these nutrients has beneficial effects on maintaining T lymphocytes, modulating the homeostasis of CD4 T-cell subpopulations, and attenuating organ injury during sepsis.

The present chapter describes the biological functions related to these specific nutrients and the physiological alterations that occur under stressed conditions. The mechanisms involved in modulating the changes of Th cell subpopulations and their favorable influences on sepsis outcomes after nutrient supplementation is also discussed.

Chapter 5 - *Objective*: To provide a narrative overview of maternal mortality in the 5-year period before and during the COVID-19 pandemic.

Material and Methods: Literature review from the PubMed/MEDLINE database. In addition, data from official institutional websites and platforms were used. The keywords used were “maternal mortality causes,” “maternal death causes,” “maternal outcomes,” “maternal mortality caused by COVID-19.” Filters were set up for the search articles in English, free full text. Sources were required to be transparent, with COVID-19 requiring laboratory confirmation and a description of the cause of death.

Results: An analysis of the scientific literature has highlighted three major aspects of maternal mortality (MM) worldwide today.

First, severe obstetric complications – bleeding, hypertensive disorders, and infections – have been the leading causes of maternal death for many years and still make a significant contribution. However, these severe obstetric complications are currently most relevant in low-income countries, where a large proportion of pregnant women are still without proper antenatal care and traditional home births persist. Severe obstetric complications are the cause of MM in sub-Saharan Africa and South Asia: bleeding (OR 28.8; 95% CI 20.3–40.7), preeclampsia or eclampsia (OR 9.13; 95% CI 6.10–13.7), infections in the mother in the antenatal period (OR 2.80; 95% CI 1.63–4.80). In Ethiopia,

MM is the result of obstructed home delivery in 17.27%, and uterine rupture is the cause of death in 7.75% of women.

Secondly, the COVID-19 pandemic since 2020 not only increases the MM ratio, but also changes the structure of the causes of MM. These changes are especially pronounced in regions with high population density. In India, the first 10 weeks of lockdown showed an increase in hospital mortality of pregnant women from 0.13% to 0.20% ($p = 0.01$). In Mexico, in 2020 compared to 2019, MM was 42.2 vs. 31.1, among the causes of MS, the proportion of respiratory diseases was 32% vs. 5.6%. In particular regions of the world, MM due to COVID-19 reached extra high values above 3399 per 100,000 live births and increased the MM rate in general.

Thirdly, there is a clear relationship between MM indicators and the income level of the population. Heart and vascular diseases (pericarditis, myocardial infarction, thromboembolism) have a significant position among the causes of MM. The MM rate from heart disease is inversely dependent on the income level of the population. This fact is explained by the unavailability of specialized care. Cardiomyopathy is the cause of death in 4% (95% CI 2–7) of mothers in developed countries and 14% (95% CI 10–18) in developing countries. Thromboembolism-related mortality among women with a mechanical heart valve depends on the drug used to prevent thrombosis and ranges from 0.9 (95% CI 0.1–1.6) for vitamin K antagonists to 3.4 (95% CI 0–7.7) for unfractionated heparin per 100 pregnancies with a mechanical heart valve.

Chapter 6 - ACTH (adrenocorticotropic hormone) belongs to the melanocortin family of peptides, is a non-opioid peptide derived from the pro-opiomelanocortin precursor, and is involved in many physiological actions (aggressive and sexual behaviors, blood pressure, feeding, learning). ACTH binds to all melanocortin receptor subtypes (MC1R-MC5R), being the only peptide of the melanocortin family that binds to MC2R.

The ACTH/MCR system is involved in nervous system alterations, food intake dysregulation, alcoholism as well as in neuroendocrine cancers (NETs) (neuroendocrine neoplasms, medullary thyroid carcinoma, pheochromocytoma) and non-neuroendocrine cancers (NNETs) (glioblastoma, melanoma, colon cancer, breast cancer, prostate cancer). MCRs are involved in the activation of metabolic pathways (cAMP, mTOR/Akt) and their synthesis is mediated by the alpha subunit of the G protein-coupled receptor (α-GPCR), which plays a key role in tumor cell growth and progression (proliferation, anti-apoptotic effect, angiogenesis).

The knowledge of the functions mediated by the ACTH/MCR system opens a window for biological applications in the oncological area, for which strategies have been developed aimed at changing the landscape in non-surgical treatment in some types of tumors where the ACTH/MCR system participates, for example, drug therapies, radiotherapy, combined therapies (drug-antagonists), agonists, antagonists and inhibitors. Therefore, the objective of this chapter is to highlight the involvement of the ACTH/MRC system in cancer and other pathologies.

Chapter 7 - Transient receptor potential vanilloid type 1 (TRPV1) receptors are known to play one of the key roles in the pathogenesis of migraine, a disease of a high-intensity headache that eventually may become chronic. Since the TRPV1 receptors are implicated in the sensitization of peripheral nociceptors, which determines the process of pain chronification, it is tempting to evaluate their contribution to the evolution of chronic migraine from an episodic form.

Previously, it was already found that the substitution of amino acids Ile585Val in the single nucleotide polymorphism (SNP) rs8065080 of the TRPV1 gene affects functional activity of these receptors in the way that carriers of the GG genotype have reduced pain sensitivity and normal sensitivity to non-painful stimuli, while carriers of the AA and AG genotypes demonstrate increased thermal pain sensitivity. However, despite a number of studies on the role of the TRPV1 receptor and its SNPs in various pain conditions, the rs8065080 polymorphism has not been studied in migraine, and particularly, in the aspect of its chronification.

In the current study the rs8065080 variants of the TRPV1 gene were evaluated in patients with episodic and chronic forms of migraine compared to healthy control group with the aim to identify the SNP’s possible associations with migraine chronification.

The study included 71 patients with clinically diagnosed migraine (40 episodic and 31 chronic) and 56 healthy controls. The diagnosis was established according to the criteria of the third edition of the International Classification of Headache Disorders. The rs8065080 SNP was determined by an allele-specific polymerase chain reaction.

The frequency distribution of the rs8065080 genotypes in patients with episodic migraine did not significantly differ from the control group: AA 34%, AG 53%, GG 13% and AA 38%, AG 41%, GG 21%. However, in chronic migraine patients the distribution of genotypes differed significantly from each of these groups - AA 61%, AG 39%, GG 0% ($p = 0.027$ and $p = 0.005$, respectively).

The increased occurrence of the AA genotype in the chronic migraine group is consistent with existing data on the involvement of this genotype in increased pain sensitivity, and the observed complete absence of the GG genotype can be interpreted as an association of this genotype with the protective mechanism against migraine chronification. The obtained results suggest involvement of TRPV1 receptors in the heterogeneity of migraine and the risk of its chronification.

Chapter 8 - This research presents one of the first known projects conducted within the United States to examine healthcare interactions between deaf/hard of hearing patients and Wyoming pharmacists. This was a mixed-method, cross-sectional study. Over half of patients reported the largest difficulty in communicating with pharmacists was hearing them due to masks and other conversations/background noise. All patients reported a preference for communicating face-to-face with pharmacists. Deaf/hard of hearing patients have unique communication needs pharmacists should be prepared to address. Effective pharmacist-patient communication is fundamental to ensure safe medication use and optimal health outcomes.

Chapter 1

The Characterization of Selected Fe-Containing Proteins: Ferritin, Transferrins, and Lactoferrin or Lactotransferrin – Their Molecular Structures and Biological Roles

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Abstract

Iron (Fe) plays a crucial role in living organisms' metabolism, anti-bacterial protection, oxygen transport, etc. Fe is important for life because of its wide availability – this element is one of the most popular ones on Earth. The Earth Core is also built from iron and nickel, and because of the very high pressure and temperature, both metals are present in a liquid form. A consequence of this fact is creating a magnetic field around the Earth as protection from the Solar wind (ionized particles and γ-radiation emitted by the Sun). Humans also use iron for building and vehicle construction, engineering, electronics, and others.

The importance of Fe for living organisms, including the human, is related to the presence of Fe ions in selected proteins like ferritin, transferrins, and lactoferrin or lactotransferrin. These proteins play a crucial role in, for instance, the protection of the body from oxidative damage and the immune system of the body. Also, a metalloprotein like hemoglobin, built of prosthetic group Fe-containing, and proteins chains, is the species responsible for oxygen transport to tissues. The importance of Fe is also related to the presence of redox-pair like $Fe^{2+} \rightleftharpoons Fe^{3+} + e^{-}$ (E = 0.77 V), and this reaction is crucial, for instance, in oxygen releasing

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by hemoglobin in tissues: 4 Fe^{2+} + O_2 → 4 Fe^{3+} + 2O^{2-}. Human iron requirements are variable and depend on age, sex, and condition of the body (from 1 mg/day in men to 2 mg in women; however, during pregnancy and lactation, it should be approx. 3 mg/day). Fe deficit can cause an iron deficiency anemia. Measuring the Fe/ferritin level is used in determining anemia. High amounts of Fe(II) are toxic, and on the opposite, Fe(III-VI) compounds are not because they are not absorbed.

In this chapter, also Fe-S protein is discussed, which are proteins Fe-S clusters containing and connected via sulfur atoms.

Keywords: Fe-containing proteins, ferritin, transferrin, lactoferrin, lactotransferrin

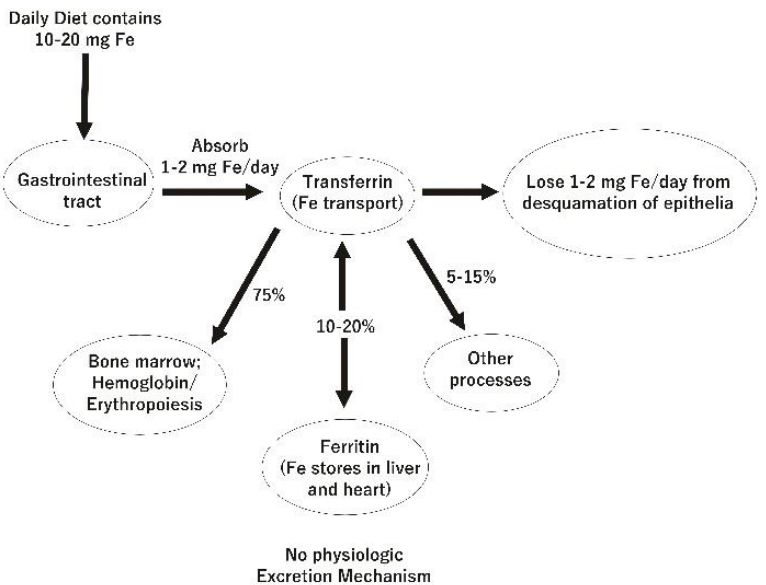
Introduction

The text below describes three proteins Fe-containing: ferritin, transferrins (a group of glycoproteins), and lactoferrin or lactotransferrin. Unlike hemoglobin, they don't contain the hem group, and a Fe ion is connected directly with amino acid residues. Also, these proteins are found with Fe ions (Fe^{2+}/Fe^{3+}) or without them. Characterization of these Fe-containing proteins, responsible for Fe transport and anti-bacterial properties, is listed in Table 1. Fe transport from the gastrointestinal tract, and its transformation into transferrins, and ferritin in the next step, are presented in Scheme 1.

Table 1. Characterization of selected Fe-containing proteins

	Ferritin	Transferrins	Lactoferrin or lactotransferrin
Description	A universal intracellular protein	A group of glycoproteins	A multifunctional protein similar to transferrin; A type of transferrin
Found	As a cytosolic protein in most tissues	In serum (in humans)	In many secretory fluids, like tears, saliva, milk, And nasal secretions; in humans, also in colostrum
Structure	A hollow globular protein built of 24 subunits	A polypeptide chain built of 679 amino acids residues (α-helices and β-sheets) and two carbohydrate chains (in humans)	One polypeptide chain

	Ferritin	Transferrins	Lactoferrin or lactotransferrin
Molecular mass [kDa]	474	80	80
Function	Fe storage in a non-toxic form, Fe releasing and controlling its level; fast Fe transporter; protection the body from oxidative damage	Fe transport through blood plasma; they have anti-bacterial properties	Antibacterial protection (in human infants and other mammals); plays a crucial role in the immune system of the body



Scheme 1. Fe transport from the gastrointestinal tract, and its transformation into transferrins, and ferritin in the next step.

Ferritin

Ferritin could be described as a universal intracellular protein, responsible for Fe storing and releasing (free Fe is toxic) and controlling its level. Ferritin was discovered in 1937 by the Laufberger, a French scientist, in horse spleen, as a new Fe protein, which contained 23% by dry weight of Fe [1] and by Addison et al. in human serum in 1972 [2]. This protein is

synthesized by most living organisms, from bacteria, archaea, plants, and animals, including mammals and humans. Its role may be defined as control of optimal Fe level – against Fe deficit or overload [3]. Ferritin is present as a cytosolic protein in most tissues and plays the role of Fe carrier in the serum. In a diagnostic test for Fe-deficiency anemia, serum ferritin level is used, which may be defined as an indirect marker of the whole Fe amount present in the body [4, 5]. The importance of serum ferritin is related to its being widely used in diagnosing and monitoring many illnesses associated with Fe deficiency or overload. However, the detailed role of serum ferritin remains unclear.

Ferritin is a hollow globular protein built of 24 subunits, and its mass is circa 474 kDa, and its diameter is about 8/12 nm, internal/external, respectively. Also, it is built of 24 protein subunits, creating a nanocage, where many metals–protein interactions are possible, which forms an ideal place for any metal storing [6]. It is a family of large (10–12 nm diameter), self-assembled protein cages, and it is synthesized from $Fe_2O_3 \bullet H_2O$ with up to 4500 Fe atoms in the central cavity, and its volume is estimated as 65 or 270 nm^3. For instance, nitrogen atoms from heterocyclic rings in the side chain can complex Fe ions. Also, Fe ions may create crystallites with hydroxide and phosphate ions in common. Then the ferritin molecule is comparable to the mineral ferrihydrite. The principal role of ferritin is storing Fe in a soluble and non-toxic form in prokaryotes and eukaryotes. In case of Fe absence in a ferritin molecule, this species is known as apoferritin. It was found that the similarity of ferritin in *E. coli* to human H-ferritin is 22% and only 14% identity in comparison *E. coli* BFR [7, 8]. Fe from ferritin is released mainly by lysosomes (then ferritin is degraded) [9, 10]. Ferritin is also used as fast Fe transporter, for instance, the hemolymph of the Polyplacophora [11, 12]. In plants, ferritin clusters deposited in the plastidial stroma may be divided into three categories [13]:

- F-2, scattered macromolecules
- F-3, paracrystalline
- F-4, crystalline array

Interestingly, no crucial difference was observed in ferritin genes in most vertebrates, and three introns and four exons are almost present [14, 15]. In human ferritin, introns are located between amino acid residues 14 and 15, 34 and 35, and 82 and 83; also, 100-200 untranslated bases at either end of the

combined exons were detected [16, 17]. According to Zoysa and Lee, the role of the tyrosine side chain at position 27 could probably be defined as biomineralization [18].

It is a widely present protein found in every cell type. The ferritin diameter is estimated as 0.17 nm, according to x-ray diffraction. This molecule is built of 182 amino acids, and it is 67% helical. Also, the ferritin has mainly an α-helical structure, and β-sheets one slight dominates, according to the mitochondrial ferritin's Ramachandran plot [19]. The types of subunits and their specific roles in the selected class of organisms are listed in Table 2. All ferritins must be analogous, and their primary sequence is comparable with the vertebrate H-type. Every ferritin complex may collect about 4500 iron (Fe^{3+}) ions.

Table 2. Characterization of ferritin, its structure in selected classes of organism [20, 21]

Class of organism	The ferritin subunits	Comments
Vertebrates	The subunits of two types are found: heavy (H) and light (L) with the molecular mass of 21 and 19 kDa, correspondingly	Their sequences are 50% identical
Amphibians	An additional ('M') type of ferritin was found	
Plants and bacteria	Single ferritin is present	Similar to ferritin found in vertebrate H-type
Gastropods of the genus *Lymnaea*	Two types of subunits were found in the yolk and somatic cells	
Pearl oyster (*Pinctada fucata*)	Extra subunit like the one in *Lymnaea soma*	Ferritin is combined with shell creation
The parasite *Schistosoma*	Two types were found: one in males, the next in females	

In eukaryotes, ferritin is built of two or three types of highly homologous and self-assembled subunits, known as L 'light' (20 kDa), H 'heavy' (22.8 kDa), and M 'middle' (21 kDa), independence of their molecular weight [22-24]. However, the M subunit was found only in bullfrogs, and only one subunit was detected in bacteria and archaea. It is essential that in the ferritin of eukaryotes, built of 24 subunits, catalytic oxidation of Fe(II) occurs in the presence of H and M subunits. In opposite, in bacteria and archaea, ferritin is built of 24 identical self-assembled subunits and 14 each of which has catalytic properties. It was observed that all subunits of bacterial and archaeal ferritin

and H and M subunits of eukaryotic ferritin could be described as H-type. Also ferroxidase activity was found (the iron conversion: $Fe^{2+} \rightarrow Fe^{3+}$). It is assumed that two Fe(II) bind the ferroxidase center simultaneously, and a product like the oxidized Fe(III)-O(H)-Fe(III) is created, which is present in each H-type subunits [25]. The site of the ferroxidase activity where diiron is bound is located in the middle of this molecule. After Fe oxidation, the products are metastable located in the ferroxidase center and is displaced by Fe^{2+} [26]. Ebrahami et al. proposed the unified model, in which Fe(II) is bound at the catalytic center and oxidized [27]. No ferroxidase activity was observed in the light chain of ferritin, and it is assumed that its role is combined with electron transfer across the protein cage [28, 29]. It is accepted that typical ferritin levels would be between 18–160 ng/mL (=µg/L) for females and 30–300 ng/mL (=µg/L) for males; more details in Table 3.

Table 3. Normal ferritin blood levels according to sex and age

	[ng/mL]
Neonates	25–200
Infants (1 to 5 months)	50–200
Children (6 months to 15 years)	7–140
Women	18–160
Men	18–270

In vertebrates, selected ferritin complexes are hetero-oligomers which could be defined as two highly related gene products whose physiological functions are slightly dissimilar. The comparable expression levels of the two genes influence the ratio of the two homologous proteins. According to Levi et al., human mitochondrial ferritin, MtF, is expressed as a pro-protein after being taken up by a mitochondrion. It is processed into a mature protein comparable to the ferritins present in the cytoplasm [30]. It is assumed that there are no introns in its genetic code, contrary to other human ferritins. Then ferritins can create their available shells.

The principal function of ferritin is Fe storage in a non-toxic form and Fe transport in case of its demand [31]. It is essential that ferritin's detailed structure and function could differ, independence of the cell types. This process is controlled by messenger RNA (mRNA), its amount, and stability. However, it may be changed because the storage and transcription of mRNA could have different efficiencies. The Fe presence is mostly an impulse for many ferritins synthesis, but an exception is observed (the Fe presence is not needed) in the yolk of *Lymnaea* sp. The main reason for Fe toxify is the redox

pair presence of Fe^{3+}/Fe^{2+} (Fe is a catalyzer), which can create hydroxyl radicals from hydrogen peroxide, according to the Fenton reaction [32].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^- \quad (1)$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+ \quad (2)$$
$$2\ H_2O_2 \rightarrow HO\bullet + HOO\bullet + H_2O \quad \text{(net reaction: 1+2)}$$

Hydrogen peroxide creates a hydroxyl radical (HO•) and a hydroxide ion (OH^-) in the presence of Fe^{2+} in the first step (Fe^{2+} is oxidized to Fe^{3+}) (1). Next, Fe^{3+} with a second hydrogen peroxide molecule creates hydroperoxyl radical (HOO•) and a proton (Fe^{3+} is reduced to Fe^{2+}) (2). The net effect of (1) and (2) reactions is the creation of two different oxygen-radical species (HO• and HOO•) and water as a byproduct. The reactions (1) and (2) are rapid and exothermic. Both free radicals are aggressive and non-selective oxidants and can oxidize many organic compounds (proteins, hydrocarbons, fats, or nucleic acids). This reaction was described first in 1935 by Haber and Weiss [33].

It is assumed that the Fe solubility became problematic circa 2.5 billion years ago when water molecules became a source of hydrogen for photosynthesis [34, 35]. Fe is toxic as most transition metals. Still, we can say that most organisms can lower Fe toxify by complexation with ferritin. In general, vertebrates have developed mechanisms to protect Fe toxify and Fe binding in many tissue or different species. Except for ferritin, also complex hemosiderin is formed. Fe is oxidized ($Fe^{2+} \rightarrow Fe^{3+}$) before complexation with apoferritin (ferritin is a product). The reticuloendothelial system is responsible for ferritin accumulation. Total body stores of Fe must correspond with the ferritin level in the blood serum; therefore, it is possible to use the serum ferritin FR5Rl as the appropriate laboratory test for estimation of Fe deposits.

Interestingly, ferritin is deposited in the shells of organisms like mollusks to regulate the Fe distribution and concentration [36-39]. They can include Fe into mineralized deposits within the teeth of their tongue-like radula. The mentioned deposits include magnetite (Fe_3O_4) and, in selected organisms, lepidocrocite (γ-FeOOH). They are used to harden the teeth.

The higher ferritin level could be an indicator in:

- Immune response: Its concentration is increased radically in case of an infection or cancer. The gene coding for ferritin is up-regulated by endotoxins, which increases the ferritin concentration. However, an inversed reaction is observed, and after infection, plasma ferritin levels are lowered; an example is organisms such as *Pseudomonas*.

Blocking Fe use is a way to destroy of metabolism of the infective agent; more precisely, the Fe concentration is reduced to around 10^{-18} M to stall bacterial growth [40-42];

- Ferritin is found mainly within cells, and it is also present in the plasma in lower concentration;
- As mentioned above, a serum ferritin level is a marker in the diagnosis of iron deficiency anemia and is measured in medical laboratories. This ferritin level direct corresponds with the total amount of iron stored in the body. However, higher than usual the ferritin level could be caused by chronic disease, and then this level is not a marker for iron overload;
- Stress response, as a response to stresses such as anoxia, as a metal chelator in *L. littorea* [43, 44]; then ferritin could be defined as an acute-phase protein that in animals, Fe sequestration is an ancient host defense response [45, 46].

It was found many roles which play mitochondrial ferritin, mostly are participation in:

- Ferroxidase/oxidoreductase activity
- $Fe^{3+/}$Fe ion/metal/transition metal binding
- Oxidation-reduction process
- Fe ions transport across membranes
- Cellular Fe ion homeostasis

It is worth mentioning that Fe storage is not the only function of ferritin. This species may protect the body from oxidative damage. Bottke et al. observed that ferritin is the primary protein component of the egg yolk in *Lymnaea stagnalis* L. and *Planorbarius corneus* L. [47, 48]. However, this ferritin has a changed genetic sequence compared to the somatic ferritin. The species are synthesized in the midgut glands, secreted into the hemolymph, and transported to the eggs.

Iron overload, also known as hemochromatosis, is a form of Fe accumulation in the body for any reason. The most important may cause hereditary hemochromatosis (HHC), a genetic disorder, and transfusional Fe overload. This illness could be caused by repeated blood transfusions [49, 50]. The stages and progression of hemochromatosis were identified at a

compromise conference of the European Association for the Study of Liver Diseases in 2000 [51]. These stages are:

- *Stage 1*: Found in patients with the genetic disorder and absence of higher Fe stores (they have 'genetic susceptibility');
- *Stage 2*: Found in patients with the genetic disorder in which phenotypic evidence of Fe overload is found; tissue or organ damage is not observed;
- *Stage 3*: In patients with the genetic disorder of Fe overload, tissue or organ damage is observed caused by high iron deposition.

Ferritin also plays other functions, for example, in materials science, carbon nanotube synthesis using iron nanoparticles, and in chemical vapor deposition. In detail, ferritin and mini-ferritins (Dps) proteins create cavities, which could be used for metal nanoparticles (NPs) synthesis as the reaction chamber [52-55]. For limitation of particle growth and as a coating, protein shells as a template are used. Also, they are required to stop coagulation/ aggregation between NPs. Let us create NPs of various sizes.

An abnormal ferritin level – lower and higher is observed when:

1. The ferritin level is increased:
    - It could be caused by Fe excess or ferritin mobilization because of an acute inflammatory reaction; it is observed a high ferritin level and average Fe level;
    - Fe overload disorders are observed, for instance, hemosiderosis or hemochromatosis (both are related to Fe accumulation in the body from any cause);
    - It could suggest the presence of adult-onset Still's disease, some porphyrias, and hemophagocytic lymphohistiocytosis/macrophage activation syndrome;
    - Any disease may cause it, and for the elimination of a higher ferritin level by acute phase reactions, using a normal C-reactive protein is recommended;
    - In patients with anorexia nervosa (mostly post-menarchal adolescent females), higher ferritin is observed in periods of acute malnourishment. Then the number of erythrocytes is decreased and caused by Fe storage as intravascular volume [56, 57]. This disease has a catabolic nature, and it is suggested that

isoferritins may be released, which may increase ferritin concentration. This sickness comes from higher Fe storage secondary to the contraction of the circulating blood volume and lower Fe losses from (secondary) amenorrhoea. In treatment, observed lower ferritin level is caused by hemopoiesis needed to fill the higher blood volume related to weight gain. In consequence, the hemoglobin and hematocrit levels are normalized;

- An increased ferritin level/Fe concentration could correspond with worse clinical outcomes in some patients with COVID-19, caused by Severe Acute Respiratory Syndrome- Coronavirus-2 [58]. The SARS-CoV-2 infection was detected first in Wuhan in China in late December 2019 and extent globally, causing the present pandemic [59];
- If ferritin level is measured using immunoassay or immuno-turbidimetric methods, isoferritins could also be detected, and an actual iron storage status may be increased [60, 61].

2. The ferritin level is decreased:
    - It could be a marker of the Fe deficit and anemia possibility. The most sensitive test for Fe deficiency anemia is the detection of low serum ferritin [62]. Nevertheless is not fully specific because the higher ferritin level could be caused by chronic inflammation. Then, a patient with a low ferritin level in the normal range could have a ferritin level. We can say that decreased ferritin levels bring more information in comparison to the normal ones;
    - It could be an indicator of a lack of vitamin C, celiac disease, or hypothyroidism;
    - Vegetarians have mostly normal serum ferritin levels, according to the opinion of the American Dietetic Association 2009. In detail, incidence of Fe-deficiency anemia among vegetarians is comparable to that of non-vegetarians. Despite vegetarian adults having lower Fe stores than non-vegetarians, their serum ferritin levels are usually within the normal range [63];
    - In some patients with restless legs syndrome (RLS), causes omnia in teenagers [64-67]. RLS is not caused by anemia, but a low Fe level could cause anemia; RLS could be defined as a sleep-related disorder, and irregular sensations in the legs at rest are observed, coupled with a need to move the affected legs [68];

- Very low blood ferritin is observed only occasionally, and it is caused mostly by a failure of the measuring tools [69].

Transferrins

Transferrins (Tf) are a group of glycoproteins in vertebrates and are responsible for the Fe transport through blood plasma [70]. Schade and Caroline isolated in 1946 the Fe binding protein with anti-bacterial properties in found human serum, known as serotransferrin [71]. Laurell and Ingelman identified the 'red' protein from pig plasma [72]. This protein was named 'transferrin', and this term replaced the name siderophilin, used for proteins Fe-containing (siderophores – from the Greek 'Fe bearers') [73]. They contain two high-affinity binding sites for Fe3+ atoms and are synthesized in the liver as an 80 kDa glycoprotein [74, 75] (transferrin has around 80 kDa). This porcine protein crystallizes in the space group *C2*, with unit-cell parameters a = 223.8, b = 44.9, c = 78.9 Å, β = 105.4° and the asymmetric unit one molecule is present. This model contains 5254 protein atoms, two Fe^{3+} cations, two CO_3^{2-} anions, one N-acetyl glucosamine moiety, and 494 water molecules. The three major groups' crystallographic structures of transferrins have been described [76].

Tf-bound Fe has coupled through Tf receptor (TfR) 1-mediated endocytosis [77]. The Tf gene encodes human transferrin, and it is located in chromosome band 3q21, consistent with the linkage of the *Tf, Tf* receptor, and melanoma p97 loci [78]. Human Tf is mainly synthesized in the liver and its half-life of about eight days in the serum [79]. Dewan et al. postulated that uptake of the Fe^{3+}-transferrin complex into an acidic endosome (viz., pH – 5.0) has occurred via receptor-mediated endocytosis. Also, the protonation of both lysine residues are observed in consequence [80]. Fe binding with transferrin glycoproteins could be described as a strong but reversible process. Fe bound to transferrin must be the most vital iron pool, and its rate of turnover (25 mg/24 h), despite it consisting of only < 0.1% (4 mg) of total Fe in the human body.

The association constant of the affinity of transferrin for Fe^{3+} is 10^{20} M^{-1} at pH 7.4; however, its value is lowered progressively if the pH is neutral or acidic [81]. It is important that transferrins may bind Fe and other transition metal ions, for instance, terbium [82, 83]. Transferrin without Fe ions is known as 'apotransferrin' [84]. Similar glycoproteins were found in many bodily

fluids of vertebrates [85]. If a transferrin molecule Fe-containing is localized, meets a transferrin receptor on the surface of a cell, then binds to it and receptor-mediated endocytosis transfers it into the cell in a vesicle. Then, diferric hTF in the serum (pH ~7.4) binds to definite TF receptors localized on the cell surface and is internalized. Also, pH decreasing in the endosome (pH ~5.6) enables Fe release [86].

A reduction of pH of the vesicle is needed to 5.5 by hydrogen ion pumps (H^+ ATPases), and Fe ions are released as a result. For Fe release rate, many factors influence, like interactions between lobes, temperature, salt, pH, and chelator. Next, transferrin without Fe ions, bounded to the receptor with its ligand, is transferred through the endocytic cycle back to the cell surface, and the process of Fe transport is continued. It is possible to carry two ions in the ferric form (Fe^{3+}) by one transferrin molecule.

The principal role of transferrin is Fe transport to all tissues from absorption centers in the duodenum and leukocyte cell macrophages. Transferrin presence is crucial if active cell division and erythropoiesis occur [87]. According to Kwok and Richardson, the Trf family of metal-binding proteins are species like [88]:

- Lactotransferrin or lactoferrin, present in milk, in many intracellular fluids, and indefinite granules of polymorphonuclear leukocytes
- Ovotransferrin, found in the egg white
- Melanotransferrin is present in most human melanomas

According to Chung, some related species to transferrin were identified like [89]:

- Blood serotransferrin (or siderophilin, usually called transferrin)
- Lactotransferrin (lactoferrin)
- Milk transferrin
- Egg white ovotransferrin (conalbumin)
- Membrane-associated melanotransferrin

It is essential that for every Fe^{3+} ion bound to the protein, one HCO_3^{--} ion is parallel attached, and three protons are released. This reaction is described by two of the following equations:

$$Fe^{3+} + H_6Tf + HCO_3^- \leftrightarrow [Fe\text{-}H_3Tf\text{-}HCO_3]^- + 3H^+ \quad (1)$$

$$Fe^{3+} + [Fe\text{-}H_3Tf\text{-}HCO_3]^- + HCO_3^- \leftrightarrow [Fe_2\text{-}Tf\text{-}(HCO_3)_2]^{2-} + 3H^+ \quad (2)$$

Transferrin is a synthesized principal in the liver; however, other tissues and organs may produce it. For instance, transferrin is made in the brain in the choroid plexus in the ventricular system [90]. The receptor aids keep Fe homeostasis in the cells by regulating the Fe level. Serum transferrin concentration is checked in Fe deficiency and Fe overload disorders like hemochromatosis. Transferrin is a polypeptide built of 679 amino acid residues and two carbohydrate chains in humans. The protein fragment has a structure of α-helices and β-sheets, creating two domains. Also, the N- and C-terminal sequences are formed by globular lobes, and the Fe-binding site is localized between these two lobes.

The Fe ions bonded to the transferrin are the same for both lobes; they are amino acid residues of two tyrosines, one aspartic acid and one histidine. An anion is needed in the process of Fe binding, like CO_3^{2-}. The Fe-bound receptor is present in the transferrin molecule as a disulfide-linked homodimer. Every monomer is built of 760 amino acid residues in humans and contains three domains: the protease, the apical, and the helical domains. It allows ligand bonding to the transferrin, and every monomer can bind to one or two Fe ions. The form of a transferrin receptor is similar to a butterfly based on the intersection of three visibly formed domains. In humans, two central transferrin receptors are present and described as transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). Except for the fact that both structures are comparable, binding specifically to human TF is observed only for TfR1, and interaction also with bovine TF – only for TfR2.

Transferrin is related to the innate immune system, too. Interestingly, transferrin is present in the mucosa as a species for Fe binding. Low free Fe levels may limit bacterial survival in a process known as iron withholding. In inflammation, it was found that the transferrin values are lower [91, 92]. Also, the transferrin level is relatively constant in the first four decades of life, and for later ages, higher levels are observed in females than in males. Higher plasma transferrin level is observed in patients with Fe deficiency anemia, pregnant women, or oral contraceptives, which causes an increase in transferrin protein expression. A higher plasma transferrin concentration may induce a reciprocal decrease in percent transferrin Fe saturation, causing a higher total Fe binding capacity in Fe deficient states [93, 94]. Lower plasma transferrin levels may cause Fe overload diseases and protein malnutrition. Also, a rare genetic disorder has been found called atransferrinemia, an autosomal recessive metabolic disorder, defined as a lack of transferrin [95].