ISSN 0006-2979, Biochemistry (Moscow), 2014, Vol. 79, No. 10, pp. 1056-1068. © Pleiades Publishing, Ltd., 2014.
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REVIEW

Decrease in ATP Biosynthesis and Dysfunction of Biological Membranes. Two Possible Key Mechanisms of Phenoptosis

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Received April 11, 2014
Revision received May 12, 2014

Abstract—Metabolic syndrome is extremely prevalent in the world and can be considered as one of main factors leading to accelerated aging and premature death. This syndrome may be closely linked with age-related disruptions in hypothalamic–pituitary system function, which perhaps represent a trigger mechanism of development of endocrine and cardiovascular pathologies. Age-related elevation of the sensitivity threshold of the hypothalamus to regulatory signals in association with low mobility and excessive diet trigger a cascade of biochemical reactions that might be used for activation of programmed death of the organism – phenoptosis. Accumulation of fatty acids in a cell and resulting lipotoxicity include resistance to insulin and leptin, endoplasmic reticulum stress, uncoupling of oxidation and phosphorylation, and dysfunction of biological membranes. Decrease in ATP synthesis is correlated with accumulation of calcium ions in cells, dysfunction of mitochondria, and increasing apoptotic activity. Age-related activation of mTOR (which is greatly influenced by excess energy substrates) has deleterious impact on one of the main mechanisms of cell defense by which defective mitochondria are replaced: mitophagy and biogenesis of mitochondria will be suppressed, and this will increase in greater degree mitochondrial dysfunction and oxidative stress. Fatty acid-induced inflammation will increase activity of nuclear factor NF-κB, the well-known stimulator of age-related pathologies. The final stage of phenoptosis can be represented by endothelium dysfunction related with oxidative stress, insulin resistance, and the most prevalent cardiovascular pathologies.

DOI: 10.1134/S0006297914100071

Key words: fatty acids, leptin, insulin, AMP-activated protein kinase, mTOR, oxidative stress, apoptosis, phenoptosis, biomembranes dysfunction, mitochondria, endoplasmic reticulum

The same diseases are usually the causes of death of people after the completion of active growth independently of sex, residence, and social status. According to data of the World Health Organization, these are cardiovascular (insults, infarcts), oncological, and immunity disturbing and weakening diseases. Although the reasons of natural mortality are the same for all people, lifetime risk for individuals can significantly differ depending on various factors.

It looks doubtful when some authors declare that there are some one hundred independent aging theories. This looks like the declarations of Belorussian cooks that there are more than one hundred different meals with potatoes, and it is possible to meet names of such meals as "meat with potatoes and vegetables" and "vegetables with meat and potatoes" on their menu. There is a similar situation with aging theories; many of them do not contradict, but intersect and supplement each other. The Russian gerontologist Anisimov emphasizes only six theories among the diversity of "aging" theories: Harman's free radical theory, Hayflick's theory of cell (replicative) senescence, the telomere theory of Olovnikov, Dilman's elevation theory of aging, the theory of soma depletion of Kirkwood, and Skulachev's theory of phenoptosis [1]. In our opinion, the theory of phenoptosis suggested by Academician Skulachev is the most viable theory that can explain not only age-related processes, but also unite them into one complete and logical construction [2, 3].

Processes that take place under the influence of various factors in the hypothalamus, cell membranes, and mitochondria represent binding units that fasten the elements in the theory of phenoptosis. Namely, in these parts of the human body, it seems that biochemical reactions and physiological transformations that serve for development of healthy prolonged lifespan of an individual or cause their accelerating aging and death occur. This balance between healthy life and premature death is apparently maintained by a mechanism including, from

Abbreviations: ER, endoplasmic reticulum; FAs, fatty acids; FFAs, free fatty acids; LPO, lipid peroxidation; PL, phospholipid; ROS, reactive oxygen species.

one side, some level of reactive oxygen species (ROS) generated in mitochondria, and the antioxidant system that combats them – from the other side. Let us look carefully, taking into account results of recent decades, how age-related changes in the hypothalamus, cell membranes, and mitochondria linked with reduction of ATP synthesis and apoptotic activity promote initiation and development of processes leading to oxidative stress and phenoptosis.

The brightest confirmation of the phenoptosis theory is the fact that phenoptosis is a reason for premature death in the great majority of events when there is a threat to the genetic program. Various factors such as high levels of radiation, accumulation of toxic compounds, viruses, and age-related changes in tissues can disrupt gene functioning. It has been established that in old age the amount of products of oxidative damage of macromolecules, including DNA, are increased in cells [4]. It is known that apoptosis, in first turn, is induced in cells with unrepaired DNA damage: two proteins, p21 and p53, cooperatively abrogate the mitotic cycle and initiate a process of self-destruction. Elimination of such cells prevents their division and the appearance of new mutant cells that might cause the death of the organism [5, 6].

There are data regarding age-related increase in p21 expression that suppresses normal functioning of the cell cycle through binding with cyclin-dependent kinases and inhibiting their activity [7, 8]. The main guard of the genome, protein p53, maintains genetic stability and homogeneity of somatic cells. Its goal is strict control the situation when a cell with unrepaired damage must be destroyed to exclude the production of new, genetically changed cells [9]. As Professor Russell and his colleagues stated, "Apoptosis is a cell death program universally reacting to various signals that 'notify' the inexpediency of prolongation of cell life" [10].

It would be logical to suggest that these processes occur at a higher level – at the level of the organism in a population, when a similar signal "notifies about the inexpediency of prolongation of cell life" is generated [3]. In this case, phenoptosis will play the role of p53 protein to prevent reproduction of defective individuals. If nature has no mechanisms of self-elimination of hopelessly "depraved" persons, then the human population would be under constant threat of elimination due to the factors mentioned above. Skulachev described in his one work how the mechanism of phenoptosis is realized in the case when the human population is threatened. He describes how a consecutive chain of events leading to death of infected individual is triggered through ADP-ribose residue attaching to diphthamide under the influence of diphtheria toxin. Concerning diphthamide, it has no function except those that assist in accelerated death of the infected person [11].

Thus, it seems quite logical and natural that the lifespan in humans who have undergone irreversible changes for some reason is significantly reduced. This mechanism of reduction and interruption of life is used to prevent reproduction of humans with damaged genes. If the genome and DNA are in safety, then the human individual may live a long time, as someone interestingly said, "Atoms do not experience aging". Of course, it is not correct to talk about the possibility of immortality because internal factors and objective reasons restrict lifespan (as, for example, Hayflick's limit, or the second law of thermodynamics). However, to be fair, it may be noted that most people do not approach close to exhaustion of the limits and finish their life before the biological term limit.

DILMAN'S ELEVATION THEORY AND PHENOPTOSIS

Scientists noted long ago that old age of humans is associated with certain diseases that occur more often in old age. Dilman was the first who, in the second half of XXth century, studied the question of "old age" diseases coupling with aging most fully and well. According to his point of view, the processes that serve at the beginning of life for normal development of the organism in the course of time bring it death. He used his observations for development of the so-called *elevation theory of aging*, and a key role was assigned to age-related disturbances in the hypothalamus–pituitary system – the main coordinator of processes in the endocrine system [12]. According to his point of view, the main reason for aging is age-related elevation of the sensitivity threshold of the hypothalamus to regulatory signals coming from nervous system and internal secretory glands. Due to such age-related changes in hypothalamus functioning, multiple regulatory mechanisms based on the feedback principle are disturbed when secretion of some hormones and enzymes must cause suppression of others.

As a result, the levels of some compounds, e.g. such hormones as insulin and cortisol and also glucose, fatty acids, and cholesterol, increases with age. At the same time, there is decrease in physiological level for other substances (some hormones and neuromediators). All these factors – increase in level of some compounds and decrease in level of others – together create conditions for triggering a chain of biochemical reactions resulted in reduction in ATP synthesis and in oxidative stress caused by increased ROS generation in mitochondria. After that, when oxidative stress acquires the necessary power to threaten severe damage to DNA, processes leading to programmed death, phenoptosis, will begin in the organism.

Unfortunately, because of his death Dilman could not analyze the results of recent studies that confirm his theoretical suggestions regarding age-related changes in the hypothalamus. Moreover, experimental data obtained recently not only confirm his theory, but also link it with the theory of phenoptosis, combining these two leading

theories into one entity. It has been established that age-related increase in free fatty acid (FFA) concentrations in the blood may cause death of neurons in the hypothalamus through apoptosis and this, logically, disrupts the regulation of the endocrine system by the hypothalamus [13]. As Dilman correctly formulated, if FFAs and cholesterol in the beginning of individual life serves for the person's growth and development, being structural components of cells, then, after completion of the active growth period, the level of these compounds begin to increase irrepressibly, becoming a reason for development of various pathologies [12].

AMPK – RESISTANCE TO LEPTIN AND INSULIN

The adipose tissue hormone *leptin* and leptin-dependent *AMP-activated protein kinase* (AMPK) play an important role in these age-related processes. Dilman did not pay much attention to them because they were not well studied in his lifetime. Leptin is a key hormone in adipose tissue regulating fat deposition in the body [14]. According to the point of view of Roger Unger, a pioneering researcher in the field of lipotoxicity, the main role of leptin is to neutralize the negative action of fatty acids (FAs) through prevention of their accumulation in non-adipose tissues [15]. AMPK is a regulator of energetic homeostasis, and it is activated in case of ATP depletion [16]. The role of this protein kinase in the organism is very important and multi-sided. It can be said that AMPK together with ROS and mTOR is one of most promising and investigated subjects in biochemistry and gerontology. These compounds are closely linked with each other: thus, AMPK suppresses the harmful activity of mTOR in a healthy organism under conditions of moderate diet and physical training [17, 18]. In these processes, an active role is performed by the "genome guard", protein p53: in normal life style and low calorie diet, it stimulates autophagy and other useful reactions, blocking in association with AMPK increase in mTOR level [9, 19, 20]. ROS influence all processes in the organism directly or indirectly.

With assistance of AMPK that activates the enzyme carnitine-palmitoyl-transferase (CTP-1), leptin promotes FA metabolism in cells by regulating FA β-oxidation [21]. CTP-1 binds FAs with carnitine, and then they are transported into mitochondria. Under deficiency of energy substrate (fatty acids), the content of leptin begins to fall, and this leads to the sense of hunger through complex feedback mechanisms. When energy substrate is in sufficient amounts, the level of leptin is increased, and this causes the sense of satiation and FA oxidation is elevated in mitochondria [22].

When the amount of energy reserves significantly exceeds the amount necessary for normal existence of a human individual (this often occurs in aging), leptin loses its regulatory properties, and leptin resistance occurs. It has been found that the critical point for leptin level is equal to 30 ng/ml. In achieving of this level, the action of leptin on leptin-dependent regions in the brain ceases [23]. The hypothalamus stops receiving and sending signals that regulate appetite. The levels of FAs, which are oxidized in a lesser degree due to leptin resistance, begin to grow. This age-related leptin resistance always coexists with another age-related type of resistance, the resistance to another regulatory hormone – insulin. What is the first – insulin or leptin dysfunction – is not yet clear. Most likely, they are formed practically simultaneously, because they regulate the same metabolic pathways – ways of energy storage and spending, and are very tightly coupled with each other. Besides, they have a few common inhibitors, blocking insulin as well as leptin signaling pathways, such as signaling pathway suppressor 3 (SOCS3) and protein-tyrosine-phosphatase-1 (PTP1) [24, 25]. There is a common factor causing resistance to both hormones – endoplasmic reticulum stress [26, 27].

A regulatory "quartet" – leptin, insulin, FAs, and glucose – controls eating, and storage and spending of energy: insulin and leptin inform the brain about long-term reserves of energy. At the same time, FA and glucose inform the brain about short-term energy reserves [28, 29]. Infringement of functioning of this quartet is very important for aging and phenoptosis in general. Therefore, we will consider associated biochemical processes in detail.

Increased insulin level, occurring in case of resistance to this hormone, activates corresponding phosphatases that "switch off" through dephosphorylation three key constituents of the normal oxidation of FAs in cells – AMPK, acetyl-CoA carboxylase, and malonyl-CoA decarboxylase. The amount of malonyl-CoA that is able to block CPT-1 will be increased. In the case of inactive CPT-1, transport of fatty acids to mitochondria will be decreased, and they will be accumulated in the cytoplasm [10]. Leptin resistance will also promote these negative processes. Specific receptors mediating phosphorylation of Janus kinases will not react with leptin. Normally these kinases phosphorylate the specific intracellular messenger – "signal conductor and transcription activator". This messenger increases activity of CPT-1 and acyl-CoA-oxidase and of coactivator 1-receptor activated by peroxisome proliferators (PGC-1α). Their low activity caused by leptin resistance will also lead to accumulation of FAs in cells [30].

LIPOTOXICITY, MITOCHONDRIAL DYSFUNCTION, AND DECREASE IN ATP SYNTHESIS

Finally, insulin and leptin resistance being stabilized for long time will cause a stable increase in FFA level and

their subsequent accumulation in non-adipose tissues – in liver, skeletal and cardiac muscles, pancreas, and thymus. These organs and tissues are very vulnerable and, in essence, defenseless in front of the negative impact of excess FAs. This excess will lead to two main consequences.

First, apoptotic processes, provided by the negative influence of metabolites of non-oxidized FAs (diacylglycerol and ceramide) and FA oxidation outside of mitochondria will increase greatly, accompanied by great production of superoxide and hydrogen peroxide [31, 32].

Mitochondrial dysfunction will force the organism to redirect FA oxidation to peroxisomes and microsomes [33]. Such improvisation will demand payment. Hydroperoxides will be formed during peroxisomal β-oxidation with participation of acyl-CoA-oxidase, transferring electrons to molecular oxygen. Enhanced ω-oxidation in microsomes is accompanied by increased production of ROS due to flavoprotein-mediated electron transfer to oxygen molecules [34, 35]. In addition, dicarbonic acid – an ω-oxidation metabolite – will lead to enhanced mitochondrial dysfunction and ROS generation, because it is able to decrease oxidative phosphorylation by disruption of the proton gradient in mitochondria [36].

Second, a paradoxical situation will occur: there is a large (sometimes, vast) deposit of energy substrates (FA and glucose), but cells experience constant energy deficiency due to disruption of ATP synthesis. This disruption of ATP synthesis, caused by FA excess, is an established fact [37].

The reason is that FAs are powerful factors that initiate the process of oxidation and uncoupling of phosphorylation. This proceeds in the following manner. It is well known that fat people readily adapt to cold weather, better than the opposite climatic event – hot weather. The fatty-dependent mechanism of respiration and uncoupling of phosphorylation is a normal physiological process called to prevent the organism from freezing – it helps the organism easily manage cold weather. If the temperature of the environment decreases, then lipase is activated and lipolysis, i.e. FA releasing out of fat deposits in the organism, is started. In this case (when it is necessary to produce more heat in competition with ATP synthesis), FAs are called to increase proton permeability of the mitochondrial membrane to deplete the proton potential immediately after its formation, thus transformation into heat instead their utilization for ATP synthesis. The following sequence of events is characteristic for this adaptive process: a fatty acid anion ($RCOO^-$) accepts H^+ (that was pumped out of mitochondria by the respiratory chain enzymes) at the outer surface of the mitochondrial membrane; then there is diffusion of protonated fatty acid (RCOOH) to inner surface of the mitochondrial membrane; then RCOOH dissociates to $RCOO^-$ and H^+ inside the mitochondria; and final stage – transfer of the $RCOO^-$ group through the ATP/ADP-antiporter or uncoupling protein to the outer surface of the mitochondrial membrane [38].

When the amounts of FAs in the organism exceed the norms envisaged by Nature, then the useful adaptive mechanism of thermoregulation transforms into a constant factor that leads to deficiency of energy due to decrease in ATP synthesis. All this is because the lipolysis that is normally suppressed by insulin is enhanced in adipose tissue cells, adipocytes, due to their insulin resistance with subsequent constant release of FAs from fat deposits. Such enhanced release leads to increase in FFA content in the inner mitochondrial membrane, which promotes uncoupling of oxidation and phosphorylation and mitochondrial dysfunction [37, 39].

This is confirmed by studies of skeletal and cardiac muscles of overweight people for whom a great decrease in mitochondrial function is characteristic: decrease in their number and disruption of biogenesis, and increase in number of mutations of mtDNA [40]. A similar picture is observed in aged people. According to data by Ozawa, after the age of 90 years deficiency of normal mitochondria in heart tissue may reach 90% [41]. Accumulation of fibers in skeletal muscles where there is no activity of cytochrome *c* oxidase (complex IV) is observed [42]. In general, it may be said that the aging process is closely linked with mitochondrial dysfunction [43].

Endoplasmic reticulum (ER) stress is another factor that promotes dysfunction of mitochondria and decrease in ATP synthesis. Again, FFA excess serves as the initial point of the process: their excessive accumulation leads to damage to the ER. Saturated fatty acids can disrupt functions of chaperones, proteins that maintain normal repair of damaged proteins and their complexes [44]. In the absence of adequate chaperone functioning, accumulation of incorrectly folded proteins occurs. An adaptive reaction – so-called unfolded protein response (UPR) – will arise in response to this. In case of low and medium level of ER stress, UPR will save the cell from death, and in high level for long time – will direct it to apoptosis [45, 46].

During its adaptive response, UPR activates three kinases clustered at the ER membrane: ATF6, PERK, and IRE 1α [47]. Their function is considered elimination of accumulated proteins with incorrectly formed structure. If the rate of formation of defective proteins exceeds the possibility of their utilization, and activation of kinase is prolonged and becomes excessive, then the biochemical cascade of apoptotic cell death will arise. Jun-dependent protein kinase (JNK) and a protein regulator of transcription CHOP (CCAAT/enhancer binding homologous protein) play the main roles in this process [48]. JNK phosphorylates proapoptotic (PUMA, Bad, Bim, and Bax) as well as antiapoptotic proteins (Bcl-2 and Bcl-xL), increasing activity of the former and decreasing activity of the latter. In addition, JNK will

increase expression of Fas-ligand and ligand TRAIL that are localized at the membrane [49, 50].

Thus, JNK may bring to cell a double "mortal" stroke: through mitochondria-dependent apoptosis and apoptosis caused by external stimuli. A similar apoptotic impact under ER stress is also characteristic for transcription factor CHOP [51]. Nevertheless, even if ER stress will not be so strong as to lead to apoptosis, calcium ion release from ER to cytoplasm will lead to dysfunction of mitochondria and decrease in ATP synthesis [52].

Age-related reduction in ATP synthesis in the heart muscle occurs according to another scenario: excess of FFAs and acetyl-CoA will block normal proceeding of the *pyruvate dehydrogenase reaction*, and uncouple processes of glycolysis, oxidative decarboxylation, and the citric acid cycle. Under hypoxia, common for aged people and various pathologies, breaking of the most favorable energy supply for the heart – aerobic oxidation of glucose – will be observed. This will lead to forced transition of energy production to the less effective anaerobic mechanism without oxygen consumption with formation of lactic acid as a final product. Deficiency of acetyl-CoA, the key substrate molecule for ATP production, caused by breaking of pyruvate dehydrogenase reaction and glycolysis and citric acid cycle uncoupling, will make the organism utilize FFAs as the main substrate [53].

Such replacement of substrates will lead to the development of intracellular acidosis, caused by accumulation of lactate and hydrogen ions in cells. In turn, intracellular acidosis leads to activation of the H^+/Na^+-pump and subsequent accumulation of sodium ions in the cytosol. In response the Na^+/Ca^{2+}-pump will be switched on and intracellular excess of sodium ions will be replaced with intracellular excess of calcium ions. Elevation of calcium ion content will provoke arrhythmia due to the disruption of cardiomyocyte contraction and relaxation, and also will lead to damage to mitochondrial membranes through phospholipase A2 activation (this will be considered below in detail) [54]. In addition, this excess of calcium ions can become a reason for so-called "stunning" of the myocardium – postponed restoration of contractile function of the myocardium after acute disruption of the coronary bloodstream [55]. To date it is known that ROS play a major negative role in the "stunning" of the myocardium [56, 57]. As a result, more reduction in ATP synthesis, progression of ischemia, and mitochondrial damages, and thus increase in apoptotic processes will be observed. This pathological mechanism was named the "calcium paradox" [53].

Cholesterol is another age-related pathogenic factor that negatively influences ATP synthesis: while accumulating with age in erythrocyte membranes, cholesterol makes it denser and makes oxygen penetration more difficult, and this weakens the main oxygen-carrying function of erythrocytes [58]. Elevation of glycosylated hemoglobin in erythrocytes that is often observed in aged people also negatively influences its oxygen-carrying function [59].

It is well known that development of pathological processes in the organism is characterized by formation of "vicious circles" (and also chain reactions), when one disruption cause another one, which, in turn, enhances and aggravates the first one, giving rise to new problems. Similar events take place in case of age-related dysfunction of mitochondria: being developed due to excessive FA and cholesterol accumulation, such dysfunction, in turn, leads to more accumulation of fatty acids and increase in oxidative stress due to disruption of their normal β-oxidation.

Dilman indicated rightly in his works that uncontrolled and unnatural elevation of appetite is observed in old age, and this is linked, in addition to leptin resistance described above, with reduction of ATP synthesis in liver cells. At the same time, the level of ATP synthesis acts like the main indicator of the energy status in the organism [12]. Moreover, this abnormal appetite increase is under the influence of age-related decrease in levels of neuromediators. As a result, disturbance in regulation of appetite by the hypothalamus occurs: age-related reduction in physical activity does not lead to appetite reduction (that must be in norm), but, on the contrary, it leads to increase in appetite [1].

Together with decrease in ATP synthesis and increase in appetite, *gluconeogenesis* is activated in the liver. When age-related or pathological resistance to insulin arises in skeletal muscles, the main consumers of glucose, cells begin to inform the brain about energy deficiency. In response to this, glucose synthesis occurs by alternative means, including, for example, use of lymphocyte proteins is initiated in the liver. Such weakening of immunity due to utilization of lymphocyte proteins undoubtedly does not proceed at no cost, and this in association with age-related involution of the main immune system organ, the thymus, will lead with time to development of severe immunodepressive states [12]. Moreover, it is well known that active gluconeogenesis is a reason for hyperglycemia that occurs on an empty stomach independently of eating [60].

Age-related disruption of ATP synthesis caused by uncoupling of oxidation and phosphorylation and by ER stress will cause the greater dysfunction of mitochondria. If constant deficiency of energy will be established, then functioning of ion channels that pump out sodium and calcium ions will be interrupted. Then increase in calcium ion content inside cells leads to activation of *phospholipase A2* localized in the inner mitochondrial membrane. The physiological role of phospholipase A2 is removal from the membrane of phospholipids oxidized by ROS. Then phospholipase A2, after activation by calcium ions, can lead to degradation of mitochondria. The enzyme cleaves mitochondrial membrane phospholipids, and the membrane loses its physiological properties due to dam-

age to the lipid bilayer: pores are formed and cations of the surrounding space will flood into mitochondria. In addition to cations, there are unnatural accumulation of phosphates and water inside mitochondria. Such accumulation of liquid can lead to swelling and breakage of mitochondrial membrane that, logically, will lead to death of mitochondria [61].

Formation of mitochondrial apoptotic pores (MAP) with participation of Bax and Bid (due to activation of calpains) and Bad and Bik (due to activation of calcineurins) proteins represents another consequence of intracellular accumulation of calcium ions. Such formation of mitochondrial pores will be a reason for the necrotic form of programmed cell death. Through activation of calcium-sensitive nitrosynthase isoform, calcium ions can lead to the development of oxidative stress [39, 52].

BIOLOGICAL MEMBRANE DYSFUNCTION AND APOPTOSIS

As been noted above, pathological changes in erythrocyte membranes contribute to age-related decrease in ATP synthesis. It is necessary to note here that all age-related changes in membranes of almost all cells in the human body are very similar to each other because they are caused by the same reasons: increase in cholesterol level and replacement of unsaturated fatty acids by saturated ones in membrane phospholipids (PLs) [62, 63]. Such changes always lead to the same consequence: accumulation of cholesterol and enrichment of PLs with saturated FAs in the membrane increases its microviscosity, and this disrupts the signaling cascades inside cells [64]. Simply, more crude and dense membrane becomes less permeable for signaling of various hormones that regulate intracellular processes. The cell becomes "deaf", and such a state is the first step toward programmed death – apoptosis. Let us consider in detail how age-related pathological changes develop in cell membranes.

All cells and intracellular structures – nuclei, mitochondria, peroxisomes, phagosomes, synaptosomes, lysosomes, endoplasmic reticulum, and Golgi apparatus – are enclosed by biological membranes. A common property of all membranes is the lipid bilayer whose main task is to serve as a barrier against penetration into a cell or organelle of ions or molecules and serve as a structural basis for functioning of multiple receptors and enzymes that participate in regulation of intracellular processes. Protein molecules incorporated into the lipid bilayer function like peculiar locks form so-called *selective channels* that are used for selective passage of some ions and molecules across the membrane. In addition, intrinsic membrane proteins serve as ion pumps that maintain the required ionic balance between the cell and the extracellular environment to provide intracellular regulation and exchange by signals in the form of electric impulses between cells [61].

Each type of a membrane differs in its particular set of proteins – enzymes and receptors. These proteins are incorporated into the lipid bilayer like a matrix, and they provide many different and important life functions: ATP formation, hormone-mediated activation of intracellular signaling pathways, recognition of foreign proteins etc. It is not be a large exaggeration to say that all the main biochemical processes in a cell and in the human body are regulated with the involvement of membranes. There are three main components of the membrane lipid bilayer: phospholipids, sphingomyelins, and cholesterol. Phospholipids, in which the main age-related changes proceed, are classified into five main types: phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and cardiolipin. They all are composed of glycerol, two fatty acids, and phosphoric acid and of individual for each phospholipid "additive": choline, serine, ethanolamine, inositol, etc. [65].

The following features are characteristic for age-related and pathological changes in erythrocyte membranes: a) increase in cholesterol and lysophosphatidylcholine levels; b) decrease in arachidonic acid content and membrane PLs and simultaneous increase in saturated FA content; c) increase in lipid bilayer microviscosity; d) disruption of intermolecular interactions between proteins and lipids; e) decrease in membrane content of high molecular weight polypeptides and simultaneous increase in the content of low molecular weight proteins; f) suppression of Ca^{2+}-ATPase activity [58]. Glycosylation of proteins observed in old age and in different pathologies makes its negative contribution to the development of degenerative damage to cell membranes, including those of erythrocytes, because it can initiate an increase in ROS generation leading to oxidative damage to membrane proteins and lipids. Namely, oxidative stress and lipid peroxidation (LPO) are the reasons for substitution of unsaturated FAs with saturated FAs in PL structures and accumulation of cholesterol in the cell membrane [66].

Age-related decrease in ATP synthesis together with oxidative stress constitute the reason for decrease in activity of *ATP-dependent aminophospholipid translocases* that leads to transformation of lipid asymmetry of the cell membrane bilayer and externalization of phosphatidylserine on the cell surface [67]. Disruption of ion balance, described above, and increase in intracellular concentration of calcium ions can activate *Ca^{2+}-dependent scramblase*, which also leads to increased expression of phosphatidylserine on the outer side of the membrane bilayer [68]. These disruptions can act as a signal to begin phagocytosis. Phosphatidylserine in cooperation with leukocytes, which recognize it at the outer membrane of a cell, directs the cell into forced apoptosis [58]. This damaging scenario is realized through specific compounds released by leukocytes – perforins and

granzymes. This apoptogenic mechanism resembles a cannon shot by a cumulative missile that burns the armor of the tank crew and blows up inside it: first, perforin makes a hole in the cell membrane, and then granzymes penetrate into the cells and initiate the process of self-destruction. Therefore, Skulachev described the proapoptotic role of phosphatidylserine as "ominous" [11].

Again, we see here another turn of the "vicious circle", when initially occurring excess of FAs and deficiency of energy lead to activation of free radical oxidation that, in turn, damages membrane proteins and lipids and increasingly aggravates the deficiency of energy. Deprived of its normal microviscosity after these changes, the cell membrane becomes unable to perform physiological functions, disrupting functioning of multiple signaling pathways. Functional activity of cells and their ability for division are impaired, and the cell sensitivity to growth factors is decreased. The number of normally functioning receptors is decreased and protein structures, such as glucose transporter GLUT, that, due to increased viscosity, cannot undergo the conformational changes necessary for transfer of glucose into the cell [64].

The reasons that lead to increase in saturated FAs and cholesterol content in cell membranes have been well studied. Replacement of unsaturated FAs in PLs by more saturated ones is caused, in first turn, by excess of saturated FAs and their oxidation outside of mitochondria. Then LPO processes are activated and unsaturated FAs in membranes that are readily oxidized become the main "victims" [63]. Deficiency of essential polyene acids due to diet peculiarities (predominance of fat meat and milk products and small amount of marine products) also promotes accumulation of saturated FAs in PLs [64]. Polyene acids are utilized by cells to synthesize specific FAs and PLs, which then form in the membrane a bilayer of so-called *annular PL*, surrounding integral proteins (receptors, ionic channels, enzymes), providing them with the possibility for conformational transformations and normal functioning due to the liquid structure. As deficiency of ω-3-penta- and hexaenoic FAs arises, they will be replaced in annular PL by more saturated tetraenoic and trienoic FAs and such decrease in unsaturation will lead to dense folding of PLs and disruption of functions of integral membrane proteins [23].

Another reason for accumulation of saturated FAs in cell membranes is blockage of their active utilization by cells (with participation of apo-E/B-100 receptors). Such blockade can be observed in old age and in different pathologies [69]. Due to blocking of active utilization, saturated FAs begin to penetrate into cells passively via a few stages (hydrolysis of triglycerides in blood, FA release and their interaction with albumin, their transport into cells). As a result, saturated FAs will form in the membrane "passage points" – local sites through which passive diffusion of mono- and bivalent cations according to the concentration gradient will occur. Such diffusion leads to misbalance of cations: increase in sodium and calcium ion content and decrease in potassium and magnesium ions. As we know, excessive calcium ion inside the cell leads to activation of phospholipase A2 and death of mitochondria [61].

To counteract the undesirable accumulation of sodium and calcium ions, response reaction arises in cells. Na^+- and Ca^{2+}-ATPases are activated to pump these ions out, and synthesis of endogenous cholesterol is increased. This increase in cholesterol level is positive at first, as it provides temporary blocking of free influx of sodium and calcium ions into the cell through insertion into the membrane at the "passage points" formed by FAs, and it increase water content in them. Long-term elevation of cholesterol content in the membrane makes the membrane denser and leads to disruption of the functions of integral membrane proteins [70].

To be fair, it is necessary to say that the opposite event – excessive content of unsaturated acids in membranes, can also negatively influence cell fate and lifespan. There is a strong correlation between saturation level of FAs in membranes and the lifespans of animals: more unsaturated acids in membranes correspond with shorter lifespan of the animal. The Spanish biologist Pamplona with colleagues described a sequence that demonstrates increase in lifespan and simultaneous reduction of docosahexaenoic ω-3 FA content in membranes: mouse → rat → rabbit → human → whale [71]. This regularity is readily explainable because unsaturated FAs are the main "victims" of LPO. Thus, fatty acid composition of cell membranes in animals to whom more prolonged lifespan is characteristic demonstrates the "golden" rule: small amount of fully unsaturated docosahexaenoic 22:6 ω-3 FA and prevalence of less unsaturated linolenic 18:03 ω-3 FA creates the balance that provides protection against LPO action as well as preserves necessary membrane fluidity.

FEATURES OF NERVOUS SYSTEM AGING

The nervous system also demonstrates age-related increase in apoptotic processes and mitochondrial dysfunction. It is necessary to note that nervous system cells are the most vulnerable cells in the organism to age-related oxidative stress and decrease in ATP synthesis [72]. Such exclusive vulnerability of neurons to deficiency of energy and increase in ROS generation is due to a few circumstances. First, neuronal tissue for physiological reasons needs the greatest oxygen supply and, consequently, there is intensive oxidative metabolism, causing increased ROS generation in mitochondria of neurons. Due to the great accumulation of unsaturated fatty acids in membranes of neurons, the neurons become vulnerable to damaging action of lipid peroxidation. Because the activ-

ity of the brain tissue antioxidant system is lower than in other organs and, in addition, there is age-related decrease in activity of some enzymes of this system, it will be clear why the nervous system cells are the most sensitive to oxidative damage [73].

A few known negative factors damage neurons. It is possible to define four main factors: β-amyloid protein, τ-protein, ceramides, and lipofuscin [74]. Their levels are under great influence, first, from age-related increase in FFA levels in the blood. In this case, excessive content of saturated FAs (palmitic and stearic) in the diet is a burdening circumstance, and all these factors form a powerful stimulus for development of different neurodegenerative diseases such as Alzheimer's disease [75].

It has been established that excess palmitic acid leads to accumulation of ceramides, and due to their participation, the regulation of terminal differentiation, proliferation, and apoptosis of neurons occurs. They cause β-galactosidase expression and promote dephosphorylation of pRb and its activation, influencing cell cycle regulators, increasing concentration of inhibitors (p21/Sdi and p27/Kip1). Thus, ceramides terminate the cell cycle that, in turn, leads to activation of protein p53 and brings the cell to apoptosis [76].

Ceramide degradation leads to formation of sphingosine, which has a definite cytotoxic effect and is able to induce apoptosis as well necrosis [77]. Age-related elevation of ceramidase expression, which participates in the process of ceramides degradation, promotes to a great degree accumulation of sphingosines in old age. These negative processes obtain additional strength under the influence of excessive intake with food of saturated FAs (especially palmitic acid): saturated FAs activate ceramidase and inhibit conversion of "harmful" sphingosine into safer sphingosine-1-phosphate. This leads to age-related sphingosine accumulation in brain structures [78].

It has been shown in experiments on animals performed by Ukrainian biologists Babenko and coauthors that saturated FAs have the ability to sufficiently enhance amyloidogenesis. This increase is because saturated FAs induce synthesis of endogenous ceramides in astroglia cells, and these cells, in turn, increase secretion of proinflammatory cytokines and nitric oxide. As a result, ROS generation increases and oxidative stress occurs, which leads to increase in β-secretase BACE1 activity and activation of stress-regulated kinases (cdk5 and GSK-3) in neurons. This leads to formation of β-amyloid protein and hyperphosphorylation of τ-protein, these two main initiators of Alzheimer-like changes in neurons [79].

FAT10 GENE KNOCKOUT

Interesting data that demonstrate the close relationships between metabolic disruptions and immune response have been obtained by American biologists who obtained mice with *FAT10* gene knockout [80]. It is well known for a long time that excessive accumulation of energy substrates, FAs, in the organism will cause an inflammatory response. One reason for this is impairment of the biological function of endoecology – maintenance of "purity" of the intercellular environment. As the concentration of analytes in the intercellular environment is increased, they become biological trash for the organism, which should be removed or utilized by resident macrophages [81]. The inflammatory reaction is a response to excess of fatty acids and includes activation of Toll-like receptors (TLRs), a key component of the innate immune system. Free (non-esterified) fatty acids in case of their excess in the intercellular environment and blood plasma form non-physiological complexes with albumin, and these complexes are chemically similar to lipopolysaccharides – toxic compounds of Gram-negative bacteria. Due to this fact, TLRs react on FFA-overloaded albumin as foreign bodies and trigger the responsive inflammatory reaction [82].

Activation of TLRs in adipose tissue is a powerful stimulus for elevation of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). In addition, TLRs have a close relationship with another proinflammatory factor – NF-κB, strongly affecting its activity. In turn, TNF-α in coordination with interferon-gamma (IFN-γ) induces increase in FAT10 activity [80].

In the opinion of the authors of the investigation, the physiological functions of FAT10 have not yet been elucidated. Nevertheless, it was to trace the expressed pleiotropic action of FAT10 on general metabolism and inflammation. Because FAT10 does not exhibit activity (or very little) in normal organs and tissues, it seems that its expression is associated with development of some pathological reactions [83]. Mice with *FAT10* knockout improved their indicators on many items that caused sufficient prolongation of their life. Absence of old age sarcopenia was the first characteristic noted by Weismann and colleagues. This effect can be explained by the fact that *FAT10* knockout increased activity of many factors of normal FA β-oxidation in mitochondria: PPAR, PGC-1α, acyl-CoA oxidase, etc. [80]. As we remember, it is disruption of β-oxidation that is one of main reasons for lipotoxicity [34, 35]. It is difficult to overestimate this effect of *FAT10* knockout and, seemingly, it may be a decisive factor that promotes lifespan prolongation and the absence of such age-related deviations as insulin resistance and accumulation of FA metabolites with apoptosis activity. Despite increase in lipolysis in *FAT10*-deficient mice, there were no negative consequences, namely, due to efficient FA oxidation. On the contrary, the authors even linked the enhanced lipolysis with reduction of total fat mass in mice. It is known that the amount of fat mass correlated inversely with lifespan [84].

Surprisingly, *FAT10* knockout made an effect similar to those observed on physical trainings – AMPK activation was registered. This may be followed by a set of positive effects associated with stimulation of oxidation, biogenesis, and autophagy [85].

Much experimental data show a close relationship between fatty acid excess, aging, and mitochondrial dysfunction [86]. The latter includes impairments of two important processes, i.e. biogenesis of mitochondria and removal, i.e. *mitophagy*, of damaged organelles. This may be critical regarding theories of aging based on age-related mitochondrial dysfunction [87]. The fact that damaged mitochondria must be removed by mitophagy is one of the main arguments to be answered by opponents of "mitochondrial" theories [88].

However, critics of the mitochondrial dysfunction "vicious circle" do not take into consideration one circumstance, namely, increased age-related mTOR activity. At the same time, it is known that this protein, in general, can negatively influence not only autophagy (in particular, mitophagy), but also suppress normal biogenesis of mitochondria [89, 90]. This, this seemingly hides a reason for the fact that defective mitochondria are not removed in time and they are not replaced by new ones. It is necessary to note that this question has not been clarified yet and it requires further study.

Special attention is attracted by the influence of *FAT10* knockout on the development and intensity of inflammatory reactions. It has been revealed that the interleukin-10 (IL-10) level was increased in FAT10-deficient mice [80]. This antiinflammatory cytokine has been noted by gerontologists due to its possible geroprotective action [91]. IL-10 begins to protect the organism in the infant period. The cytokine was revealed in mother's milk, where it performs a role in protection of the newborn from inflammation. In the adult organism, IL-10 has expressed ability to suppress inflammation (simultaneously increasing insulin sensitivity): it suppresses activity of proinflammatory cytokines and NF-κB and prevents ER stress [92]. There are data indication that IL-10 can protect heart and brain from ischemia damage [93, 94].

In addition, the authors emphasize the influence of increased FAT10 expression on the development of chromosomal anomalies: mice with knockout at this gene are less subject to development of tumors in comparison with normal animals. These data suggest that FAT10 may play an important role as a regulator of "immune–metabolic homeostasis" [80].

Let us make a conclusion. Taking into account all that mentioned above, it is possible to construct the following more or less likely sequence of age-related changes leading, finally, to phenoptosis. As Dilman correctly suggested, there is disruption of metabolic processes regulated by the hypothalamus with age [12]. New data supporting this hypothesis about the key role of hypothalamus in aging appear every year. Thus, Cay and colleagues in 2013 explored relationships between age-related inflammation in the hypothalamus with activation of nuclear factor NF-κB and suppression of gonadotropin-releasing hormone synthesis [95]. This work completes a series of investigations regarding mechanisms of the influence of fatty acids on activation of NF-κB and increase in apoptotic activity in the hypothalamus [13, 96, 97]. As it can be suggested, the regulation of the endocrine system by hypothalamus is disrupted, and this is followed by increase in regulatory hormones (insulin, leptin) and energy substrate (fatty acids and glucose) levels.

Such elevation, aggravated by low mobility and excessive food consumption, will lead to resistance to leptin and insulin and accumulation of fatty acids in cells. Excess fatty acids will lead to lipotoxicity. Disruption of chaperone functioning, endoplasmic reticulum stress, and mitochondrial dysfunction will be observed. Fatty acids accumulated in mitochondrial membranes will uncouple oxidation and phosphorylation and decrease synthesis of ATP. Decrease in ATP synthesis will cause disruption the functioning of ion pumps and lead to calcium accumulation and to more mitochondrial dysfunction. These processes will be accompanied by increase in apoptotic activity in various organs and reduction in their functional abilities. Besides suppression of energy-dependent processes, decrease in ATP synthesis carries an additional danger. It was recently shown that ATP, in addition to its energy function, could act in the organism in a new role – as a signaling molecule [98]. In nervous tissue, ATP performs an especially important role as a neuromediator [99], and it is possible to suggest that its deficiency will have a negative influence on all regulatory processes.

Dysfunction of biological membranes and apoptosis-induced death of cells will proceed in parallel for the same reasons. Age-related mTOR activation, about which Blagosklonny discusses much, will lead to suppression of activity of AMPK and coactivator 1-receptor, activated by proliferators of peroxisomes (PGC-1α) that, in turn, will decrease the rate of mitochondrial biogenesis and additionally aggravate the situation [100, 101]. Moreover, as it is known that insulin resistance also negatively influences activity of AMPK, and, hence, mitophagy and biogenesis of mitochondria [102]. As a result, excess of defective mitochondria, increase in ROS generation, decrease in ATP synthesis, mitoptosis, and apoptosis should be observed.

Accumulation of fatty acid metabolites in neuronal tissue and inflammation processes induced by fatty acids through activation of cytokines, toll-like receptors, and NF-κB will increase apoptotic processes and overall dysregulation [103]. The postmitotic nature of neurons makes them subject to accumulation of various types of damages. Special attention here is attracted by lysosomal

dysfunction, i.e. lysosome overloading with lipofuscin caused by increase in ROS generation, which makes large obstacles for normal autophagy [104]. As it is presently known, it is possible to prevent age-related neurodegenerative processes by controlled diet and physical training. It was established that limitation of calories and physical training behave as effective neuroprotectors [105]. Many factors that protect neurons from damage are activated under their influence through mechanisms of hormesis: heat-shock protein Hsp70, brain neurotrophic factor, glucose-regulated protein 78 (*GRP 78*), β-oxybutyrate, etc., and also AMPK and PGC-1α [106, 107].

Endothelium dysfunction, closely linked with oxidative stress, insulin resistance, and fatty acids, becomes an initial point in the development of prevalent age-related cardiovascular pathologies that most of all complete the process of phenoptosis [108]. One more important property of fatty acids should be noted. In addition to direct negative impact on cells, they can act as epigenetic factors influencing the activities of genes. There are enough experimental data demonstrating that excessive consumption of saturated fatty acids (palmitic and stearic) negatively influences genes associated with energy processes in mitochondria and the renewal of mitochondria [109, 110].

In conclusion, after all many investigations during the last hundred years, we have come to understand that this is well known for a long time. *The basis for healthy long live is formed*, *as before*, *by moderate diet and muscle activity*. So, the insulin resistance (as the first step to accelerated aging) occurring on excessive food consumption and low mobility background must not surprise anybody if we recall that the hormone insulin originated in the phylogenetic process exclusively as a mean for providing locomotion. If an individual has the habit to eat much and use muscles only to reach the refrigerator, then no magic drugs will help them to delay old age, because they have no strength to replace adaptive mechanisms formed during million years of evolution. The mechanisms that are directed to provide for long life and reproduction of strong and healthy individuals, while weak and sick individual will perish sooner to make no threat for all the population.

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