A New Look at the Causes of Heart Failure at Old Age

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Abstract

Most of the time, researchers in different fields of Science develop certain ideas or conceptions For some time these conceptions exist separately, but from time to time something happens, and these separate ideas, like small parts of a complex puzzle, begin to fell into much larger Scientific picture. In this brief review we make an attempt to bind together recently published new data regarding age-associated mutations of mtDNA, roles of mitochondrial phospholipids cardiolipin (CL) and phosphatidylethanolamine (PEA) in mitochondrial functions, oxidation of fatty acids in the heart's energy metabolism and in oxidative stress, for better understanding of the mechanisms of aging and age-associated heart diseases. We came to conclusion that increased β-oxidation of fatty acids in people at the ages after 50 - 60 accelerates production of perhydroxyl radical, which activates in the heart the nonenzymatic autoxidation of CL and PEA and polyunsaturated fatty acids (PUFA). Autooxidation of PUFA produces a racemic mixture of hundreds of toxic molecules that gradually make mitochondria dysfunctional. Due to a large redundancy of mitochondrial enzymes these widely variable harms remain unnoticed for a long time. We conclude that not mutated mtDNA, but slowly accumulating dysfunctions cause wear and tear of the heart.

***Keywords:** mtDNA; Cardiolipin (CL); Phosphatidylethanolamine (PEA); Polyunsaturated Fatty Acids (PUFA)*

Introduction

More than 50 years ago, physiologists, working with the perfused animal hearts, have established that in the working heart up to 95% of energy is obtained by way of β-oxidation of fatty acids [1]. However, little was known how exactly this physiologically important metabolic function is fulfilled at the level of the heart mitochondria. Recently, we have shown that isolated rat heart mitochondria oxidize fatty acids at high rates in all metabolic states only in the presence of other mitochondrial substrates, such as succinate, glutamate or pyruvate, which we designated collectively as fatty acids oxidation "supporting substrates" (SS) [2,3]. For the purpose of brevity, the substrate mixtures of fatty acids and any of the supporting substrates will be designated in this review as "FA+SS". One of the most important features of fatty acids oxidation by isolated heart mitochondria in the presence of supporting substrates (FA+SS) is a manifold increase in reactive oxygen species (ROS) production in the State 4 metabolic state (resting respiration in the absence of added ADP) [2,3]. This is illustrated in figure 1.

Taking in consideration that the human heart usually works at very different workloads [4], we suggested that under conditions of low physical activity, the highly efficient oxidation of FA+SS may cause increased oxidative damages to the heart [2,3]. This is particularly important for people after ages 55 - 60, when human organisms "switch" to predominant utilization of fatty acids as the main source of
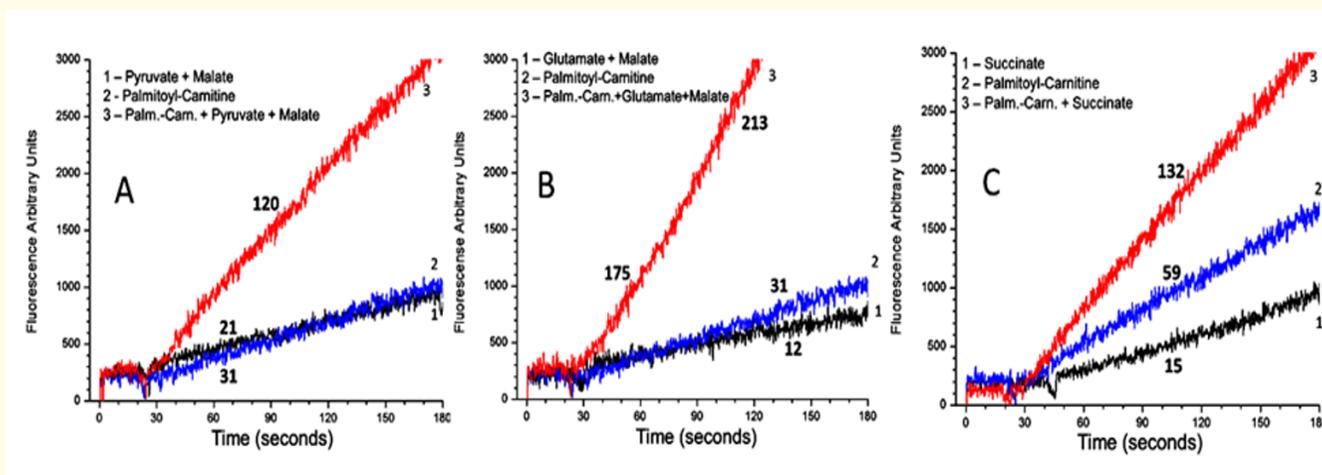
Figure 1: *Production of ROS by RHM oxidizing palmitoyl-carnitine and the effects of supporting substrates. Designations: 1. Support-ing substrate (SS) only; 2. Palmitoyl-carnitine only, and 3. Palmitoyl-carnitine + SS. Supporting substrates: Figure 1A: 2.5 mM pyruvate + 2 mM malate, Figure 1B: 5 mM glutamate + 2mM malate and Figure 1C: 5 mM succinate. The incubation medium contained: Amplex Red 2 μM, horse radish peroxidase 2 Units, substrates as indicated in Methods, volume 1 ml. The reaction was initiated by addition of 50 μg of heart mitochondria. Initial rates were measured for 3 minutes. Numbers at the traces are the rates of H_2O_2 production in picomol H_2O_2/min/mg protein RHM. The figures were taken from [2].*

energy, often accompanied by changes in the body's physical appearances and general metabolism, known to physicians as the "Metabolic syndrome" [5-7].

In this review we briefly discuss the latest developments in the field of oxidative stress and associated pathological changes in the ag-ing heart.

Aging, oxidative stress and mitochondrial dysfunctions

Harman, the founder of the free radical theory of aging, defined aging as "the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age" [8,9]. For the last 40 years the free radical theory dominated among other theories of aging and seemed most experimentally supported [10]. For a long time, the only known reliable markers of the age-associated damages caused by oxidative stress were mutations and deletions of the mitochondrial DNA (mtDNA). Accumulation of mutated mtDNA was regarded as the main cause of many diseases and attempts have been made to facilitate diagnosis of different syndromes by using methods of analysis of mitochondrial genome [11,12].

Recently, however, it was realized that the current paradigm of understanding the mechanisms of oxidative stress does not fully explain mechanisms involved in the process of aging and age-associated diseases. First, it was concluded that practically all studied radicals for several reasons cannot be responsible for mutations of mtDNA [3,13-16]. Although a large number of studies have presented compelling evidence for the key roles of mtDNA in age-related pathology, most of them are correlative rather than demonstrating cause [16]. I will not discuss here the argumentations provided by the opponents of the free radical theory of mtDNA mutation because this has been done in the recent comprehensive review by Chocron., *et al* [16]. Pinto and Moraes [13] discussed new hypotheses of age-associated mitochon-drial dysfunctions based on the roles of reactive oxygen species as signaling molecules and on their roles in mediating stress responses to

the age-dependent damages. Importantly, the authors of the cited reviews admitted that the primary causes of aging and mitochondrial dysfunctions, as well as mechanisms of mtDNA mutations, remain unknown [13-16].

Damaged mitochondrial phospholipids are specific markers of aging

Ten years ago, data have been published that oxidized cardiolipin (CL) is a specific marker of aging in mitochondria from the aged animals [17,18]. Approximately at the same time, it has also been shown that another mitochondrial phospholipid, phosphatidylethanolamine (PEA), may be damaged as the result of the isoprostane pathway of lipid peroxidation (IPLP) of polyunsaturated fatty acids (PUFA) [19,20]. Cardiolipin is a unique phospholipid, which is present exclusively in mitochondria, predominantly in the inner leaflet of the inner membrane [21]. CL bears strong negative charges at its small head and normally contains four linoleic acids with two double bonds [22].

PEA contains at C1 ethanolamine group, which forms a very small head of the phospholipid, and at C2 it normally contains a molecule of arachidonic acid in the heart (C20:4 ω6) or docosahexaenoic acid (C22:6 ω4) in the brain mitochondria. PEA is present in many cellular membranes, but the highest content is in mitochondria where it plays important role in the processes of fusion and fission [23]. Both phospholipids have strongly conical shape and therefore cannot form regular flat membranes. However, CL together with PEA, support the superstructural organization of enzymes participating in oxidative phosphorylation (OXPHOS) by allowing accommodation of the multienzyme complexes into the sharp curves of the inner membrane [24].

Thus, oxidative damages to CL and PEA result in gradual disruption of the superstructural organization of OXPHOS enzymes and other multienzyme complexes, which manifests itself in various kinds of lesions of mitochondrial functions [3,17,18]. These damages initially do not make much harm to the heart's function because of tremendous redundancy of the OXPHOS enzymes, but gradually they accumulate and result in a heart's disease.

Cardiolipin promotes formation of the protonated form of superoxide radical (HO$_2^\bullet$), which is the cause of IPLP

Approximately 30 years ago, scientists from the Vanderbilt University have discovered the nonenzymatic pathway of PUFA lipid peroxidation, which produces a racemic mixture of hundreds of products possessing enormous variations in molecular positional- and stereo- isomerism in structure and biological activities. Many of the products of IPLP resemble normal prostanoids, and thus were named isoprostanes, others are highly toxic, because they react with lipids and proteins [25]. The products of IPLP were found to be the most reliable and early markers of oxidative damages to lipids and proteins during aging and associated pathologies [26,27]. Some of the toxic products of PUFA autoxidation are γ-ketoaldehydes, and the most active among them are isolevuglandins (IsoLG). IsoLGs are so reactive, that were revealed only as adducts with lysine of proteins or ethanolamine of PEA [28]. The differences between IPLP and the "classical" lipid peroxidation have been discussed [29]. The mechanism responsible for such enormous diversity of stereo- and positional isomerism of the final products of PUFA autoxidation during the IPLP remained until recently unknown, as well as the radical responsible for initiation of IPLP.

One of the most important features of IPLP is that the putative radical reacts with a PUFA in the hydrophobic milieu of the inner mitochondrial membrane. This excludes most of the well-known radicals since they normally exist in the hydrophilic milieu, like superoxide radical (O$_2^\bullet$), which is inactivated by the hydrate shell, after being expelled from the membrane, or hydroxyl radical ($^\bullet$OH). The hydrophobic nitric oxide radical ($^\bullet$NO) is not chemically active enough to activate IPLP. The only candidate for activation of IPLP is the perhydroxyl radical (HO$_2^\bullet$) [29]. The suggested mechanism of initiation of IPLP by HO$_2^\bullet$ was presented [29]. The hypothesis is based on the data obtained by many researchers that CL forms in the inner mitochondrial membrane clusters bearing strong negative charges, which are essential for activation of the respiratory chain enzymes and ATP synthesis by promoting binding and conductance of protons through the hydrophobic milieu of the inner membrane [30-33]. At some regions of the IMM, cardiolipin creates a strong negative charge, where local pH may be several units lower than in the bulk phase of a compartment. This promotes formation of perhydroxyl radical in the reversible reaction O$_2^\bullet$ + H$^+$ ↔ HO$_2^\bullet$ with pKa = 4.88 [34]. This reaction occurs at the interface inner membrane/water, and because HO$_2^\bullet$ is highly

hydrophobic, it goes back into the lipid phase of the membrane where specifically reacts with a PUFA [34]. The details of activation IPLP by $HO_2^\bullet$ have been discussed [29].

Because the amount of perhydroxyl radical is proportional to the existing concentration of superoxide radical, the manifold increase in production of $O_2^\bullet$ observed with increased oxidation of FAs+SS at old age, will inevitably promote formation of $HO_2^\bullet$ and thus IPLP. Activation of IPLP at old age may be even further increased by the fact that the activity of superoxide dismutase 2 may be diminished due to the age-dependent loss of activity of SIRT-3 deacetylase [35,36].

The nature of the metabolic syndrome

The Harman's point of view that aging results from "the progressive accumulation of harmful changes" in cells during lifetime, evidently is too limiting for understanding the mechanisms of aging. Aging is also the process of development in time [37], when a human organism goes through a sequence of stages, from a baby to an old man or woman, the process known as postembryonic ontogenesis. Thus, from the point of view of normal ontogenesis, the development of the metabolic syndrome represents a normal process of transition from the reproductive to post-reproductive stage of an individual. Evidently, metabolic syndrome represents the last, or one of the last stages of normal ontogenesis, which were not affected much by natural selection in the course of human evolution. The type of the numerous external changes in the body's structure that occur after 50, more likely reflect the body structure of our ancestors, who lived during and after the last ice-age. Similarly, the type of metabolism after transition of an individual to the post-reproductive stage of ontogenesis, also resembles the metabolic features of our ancestors, who often starved, ate mostly meat and fat, but never consumed sweets and fast carbohydrates. We suggest that some pathological conditions, observed in people with metabolic syndrome, for example diabetes 2, to a large degree may be caused by mismatch of "contemporary lifestyle" and "ancient" metabolic phenotype.

The importance of the metabolic conditions in the heart mitochondria

Recently we have shown that the rate of ROS production during synergistic β-oxidation of fatty acids by heart mitochondria strongly depends on the type of supporting substrates [2,3]. Figure 2 illustrates how simultaneous oxidation of palmitoyl-carnitine and different supporting substrates and their mixtures affect production of ROS by the isolated rat heart mitochondria.
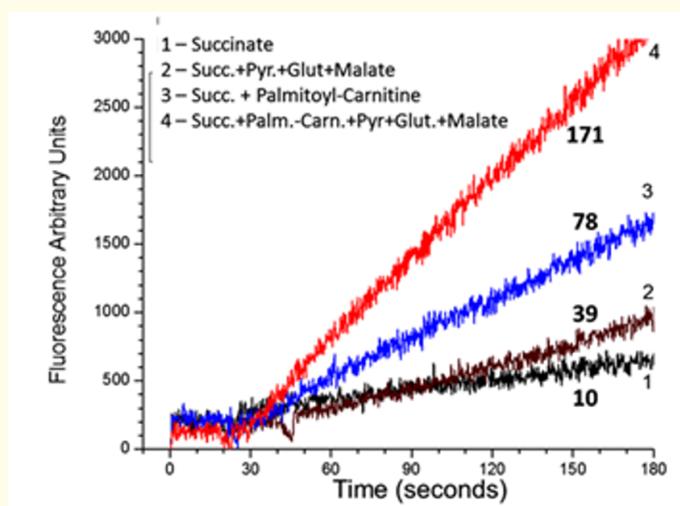

Figure 2: *Effect of various mixtures of supporting substrates on production of ROS by rat heart mitochondria oxidizing palmitoyl-carnitine. Incubation conditions as in figure 1.*

One of the important features of metabolic syndrome is a dramatic increase in utilization of fatty acids for production of energy by mitochondria, particularly in women after menopause [38,39]. From figures 1 and 2 we can suggest that in aged individuals with metabolic syndrome increased utilization of fatty acids may cause increased oxidative stress and thus accelerated rate of aging. Figure 2 also illustrates that the rate of aging may depend on the nature of the supportive substrates, which to a significant degree depends on the type of a diet. This is a new type of experimental data, which needs to be further investigated.

Conclusion

The data presented in this review in general support the free radical theory of aging, but the focus is now centered on the protonated form of superoxide radical, which previously was largely ignored. The major damaging effect of perhydroxyl radical consists in activation of the isoprostane lipid peroxidation. The numerous toxic products of the IPLP evidently cause numerous and different lesions to mitochondria gradually causing wear and tear of mitochondrial and cellular functions. There are probably two major types of direct lesions: one type of mitochondrial dysfunctions is bound with oxidation of cardiolipin and phosphatidylethanolamine, which lead to various dysfunctions of polyenzymatic complexes. The second type of lesions is caused by direct interactions of toxic products, like isolevuglandins, which directly form adducts with lysine of proteins and PEA. Evidently, to this type of damages belong damages to replication of mtDNA [15,16,40]. Anderson., *et al.* [40] have shown that exo domain of mtDNA replicase, Pol gamma, is far more sensitive to oxidation than pol domain. The authors suggested that under oxidative conditions, exonuclease activity therefore declines more rapidly than polymerase. The oxidized Pol gamma becomes editing-deficient, displaying a 20-fold elevated mutations than the unoxidized enzyme [40]. PEA may be damaged by both pathways: via autoxidation of a PUFA at C2, and via formation of adducts of ethanolamine with IsoLG produced upon activation if IPLP by $HO_2^{\bullet}$.

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