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Synergistic Oxidation of Fatty Acids, Glucose and Amino Acids Metabolites by Isolated Rat Heart Mitochondria

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Abstract

Mitochondria in the unceasingly working heart consume large amount of substrates to provide enough ATP to satisfy the energy demands. Experiments with heart perfusion established the competition for oxidation between glucose and fatty acids (FAs), and that up to 70 - 90% of ATP is produced by heart mitochondria for the expense of FAs. However, for a number of reasons, the relationships between glucose metabolites and fatty acids at the mitochondrial level remained poorly understood. We studied respiratory activities, membrane potential and ROS production by isolated rat heart mitochondria (RHM) oxidizing pyruvate (pyr), succinate (suc), glutamate (glut), palmitoyl carnitine (P-C) and their mixtures. We found that suc and P-C, when used alone, were poor substrates for RHM. However, when P-C was oxidized in the presence of suc, glut, or pyr, the respiratory rates in State 4 increased several fold, ATP production increased by 35% when compared with glut and by 230% in comparison with P-C. Thus, glucose metabolites and glut synergistically enhance oxidation of FAs. The several fold increase in State 4 respiration was the result of dramatic increase in production of superoxide radicals measured by the Amplex red method. Metabolic syndrome (MS) is accompanied by the cluster of pathologies including cardiovascular pathologies. We suggest that MS results not from aging, wrong diet or oxidative stress, but at the base, it possibly represents the final stage of the human ontogenesis. This explains the body's structural changes and the phenomenon of "metabolic dysregulation", when oxidation of FAs is not controlled by glucose metabolism. Increased fatty acids oxidation facilitates production of ROS and thus may cause accelerated aging of the heart and other organs.

Keywords: *Heart Mitochondria; Respiration; Membrane Potential; ROS Production; Metabolic Syndrome; Fatty Acids; Palmitoyl-Carnitine; Pyruvate; Glutamate*

Abbreviations

FAs: Fatty Acids; CCCP: Carbonyl Cyanide m-Chlorophenylhydrazone; CVD: Cardiovascular Diseases; IMM: Inner Mitochondrial Membrane; MS: Metabolic Syndrome; OAA: Oxaloacetate; RET: Reverse Electron Transport; RHM: Rat Heart Mitochondria; ROS: Reactive Oxygen Species; SDH: Succinate Dehydrogenase

Introduction

In a recent comprehensive review on heart mitochondria, Murphy., et al. [1] have once again stressed that "the incessant energy requirements of the heart are sustained by the consumption of a mass of ATP daily that surpasses cardiac weight itself by approximately 5 to 10 fold [2]". The energy for the continuous pumping of blood through the vascular system is provided by the large amount of mitochondria in cardiomyocytes, which constitute between 23% and 32% of the myocellular volume [3]. Heart mitochondria also have very large number of respiratory chain complexes and the matrix, which is dense and has small osmotically active volume [4]. This limits diffusion

of metabolites and promotes functioning of enzymes in the matrix and the inner mitochondrial membrane (IMM) as multienzyme complexes [4,5].

High respiratory activity of the unceasingly working heart requires constant and ample supply to mitochondria substrates and oxygen. The first information about substrates metabolic activity of the heart was obtained in the middle of the 20th century in experiments with perfusion of the isolated heart. These experiments have established the competition for oxidation between glucose and fatty acids [6-9]. In 1963 Randle., *et al.* [10] proposed the so called Glucose Fatty Acid Cycle, which depicts the reciprocal and independent metabolic relationship between glucose and fatty acids. When put simply, the essence of the glucose fatty acid cycle is this: provision of glucose promotes glucose oxidation and glucose and lipid storage, and inhibits fatty acid oxidation; provision of free fatty acids promotes fatty acid oxidation and storage, inhibits glucose oxidation and may promote glucose storage if glycogen reserves are incomplete [11]. The details of reciprocal influences of glucose and fatty acids on their metabolism in the heart were discussed in numerous papers [6-9,12,13] and reviews [10,11,14-18], of which we cite only few. The heart perfusion experiments led to conclusion that normally heart receives up to 70 - 90% of required energy from oxidation of fatty acids [19]. However, unlike substrate interactions in the whole heart [14] or body [11,17] substrate interactions in the heart mitochondria remain almost unstudied.

During the 70 years of mitochondrial research, scientists have learned very much about the structure and functions of the isolated mitochondria from various organs, including heart mitochondria. During this period, scientists usually required steadily high supply of hydrogen to the respiratory chain, which would provide high energization of mitochondria. For several reasons, which were discussed in [20], the best substrate choice became succinate in the presence of rotenone - the inhibitor of the respiratory chain Complex I. Succinate + rotenone became almost universal substrate for all types of mitochondria, including heart mitochondria [21,22].

Oxidation of succinate in the presence of rotenone provides high rates of respiration in all metabolic states with high respiratory control ratios. Succinate + rotenone was very useful for studies of many functions of mitochondria. It was also believed that succinate reflects the activity of Complex II of the respiratory chain, and in order to study the activities associated with Complex I, researchers used as substrates either glutamate, or pyruvate, usually in the presence of malate. Other substrates for mitochondrial respiration, such as α-ketoglutarate, citrate or acyl-carnitines were much less common among researchers. When the main focus of mitochondrial research shifted towards the roles mitochondria play in pathogenesis of numerous diseases, it turned out that the practice of the usage of single substrates, such as glutamate, pyruvate, and succinate + rotenone turned out ineffective for understanding the metabolic mitochondrial physiology in conjunction with the physiology of the host organ. Currently, interest to mitochondria is high, but practically there are very few studies on mitochondrial respiration.

In the current paper we use a new methodological approach to study energy metabolism of the rat heart mitochondria based on comparisons and analysis of respiratory rates of mitochondria oxidizing various substrates and their mixtures. We argue that intrinsic inhibition of succinate dehydrogenase (SDH) by endogenous oxaloacetate (OAA) is not an experimental artefact, but a natural mechanism to restrict production of ROS by mitochondria [20]. We show also that at the mitochondrial level there are not competitive, but synergistic interactions between oxidation of fatty acids and metabolites of glucose and amino acid glutamate. Synergistic oxidation of fatty acids with other mitochondrial metabolites not only greatly increases the rate of oxidative phosphorylation, but also dramatically increases production of reactive oxygen species. We discuss also the significance of our findings for understanding the metabolic syndrome and associated cardiovascular diseases.

Materials and Methods

Animals: All animal use complied with National Institutes of Health guidelines and was approved by the IACUC of the Carolinas Medical Center. Male Lewis and Sprague Dawley rats (180 - 250g) from Taconic were used for isolation of rat heart mitochondria (RHM).

Isolation of mitochondria: RHM were isolated as described in [23] and purified in Percoll gradients as described in [24]. The isolation medium contained 75 mM mannitol, 150 mM sucrose, 20 mM MOPS, pH 7.2, 1 mM EGTA. The final suspensions of mitochondria were

prepared using the incubation medium. With this medium the suspension of RHM was much less prone to clotting and sedimentation. Mitochondrial protein was determined with the Pierce Coomassie protein assay reagent kit.

Simultaneous measurement of mitochondrial respiration and membrane potential: Respiratory activities of the mitochondria were measured using a custom-made plastic minichamber of 560 µL volume equipped with a standard YSI (Yellow Spring Instrument Co., Inc.) oxygen minielectrode connected to a YSI Model 5300 Biological Oxygen Monitor, a custom-made tetraphenylphosphonium TPP^+-sensitive minielectrode, and a KCl bridge to a Ag/AgCl reference electrode connected to a pH meter as described in [23]. All instruments were connected to the data acquisition system. Since the internal volume (matrix) of heart mitochondria remains uncertain, the exact calculations of the membrane potential (ΔΨ) in mV were impossible. For this reason, the steady state values of ΔΨ in the State 4 can be done qualitatively by comparison of the degree of TPP+ consumption upon addition of mitochondria. The comparison is possible only for the same type of mitochondria.

The incubation medium contained: 125 mM KCl, 10 mM MOPS, pH 7.2, 2 mM $MgCl_2$, 2 mM KH_2PO_4, 10 mM NaCl, 1 mM EGTA, 0.7 mM $CaCl_2$. At a Ca^{2+}/EGTA ratio of 0.7, the free [Ca^{2+}] is close to 1 µM as determined using Fura-2. The substrate concentrations were: 5 mM succinate without rotenone, 5 mM glutamate, 2.5 mM pyruvate, and 2 mM malate, palmitoyl-carnitine 0.05 mM. The working solution of palmitoyl-carnitine was prepared in 50% ethanol of the analytical grade of purity. Oxidative phosphorylation (state 3) was initiated by addition of 150 µM ADP. The uncoupled respiration (state 3U) was stimulated by titration with carbonyl cyanide m-chlorophenylhydrazone (CCCP) in 0.05 µM aliquots until maximum rate of oxygen consumption was obtained. At the RHM concentration of 0.3 mg per chamber volume of 0.56 ml full uncoupling was achieved with 0.1 µM CCCP.

Measurements of hydrogen peroxide generation: H_2O_2 was determined using the Amplex red (Molecular Probes) method as described in [23-25]. The incubation medium contained: Amplex Red 2 µM, Horse radish peroxidase 2 Units, substrates as indicated above in the Methods, volume 1 ml. The reaction was initiated by addition of 50 µg of mitochondrial protein. Initial rates were measured for 3 minutes. Additions of resorufin (Sigma) were used for calibration of the fluorescence scale. The calibrations were done at the end of each experimental day. Every experiment also required the time control with mitochondria incubated without substrates, and with substrates, but without mitochondria. This was done because in a big city, such as Atlanta (GA) or Charlotte (NC), air condition (mainly ozone) affected the spontaneous accumulation of peroxides, sometimes at the rates compatible with the rate of H_2O_2 production during oxidation of glutamate or pyruvate. Sufficient for errors amount of ozone may be produced by the powerful lamp of some spectrofluorometers and filter fluorometers. Therefore all calculated rates of ROS production were corrected for this background increase in H_2O_2. Fluorometric measurements were made using a highly sensitive fluorometer from C&L Company, Middletown, Pennsylvania (www.fluorescence.com).

Data acquisition: Data acquisition was performed using hardware and software from C&L Company.

Chemicals: Chemicals were of highest purity available. All solutions were made using glass bidistilled water.

Statistics: Comparisons between two groups were made by unpaired t-test and comparisons between more than two groups were made by ANOVAs followed by post hoc tests.

Results and Discussion

Metabolic properties of heart mitochondria: It is clear that *in vivo*, there is no inhibitor of Complex I, like rotenone. However, when brain and heart mitochondria oxidize succinate in the absence of rotenone, the respiration becomes quickly repressed due to inhibition of SDH by oxaloacetate, which is a very strong inhibitor of this enzyme [18]. Figure 1A illustrates this situation with RHM oxidizing succinate in the absence of rotenone. During the resting respiration (State 4), RHM consume significantly more O2 as compared with the RHM oxidizing succinate + rotenone (not shown). However, upon addition of ADP (metabolic State 3) the respiration becomes quickly inhibited, and the uncoupler suppresses respiration even further (State 3U). This inhibition was instantly released by addition of 5 mM glutamate

(Figure 1A). Figure 1B shows that when RHM oxidize the mixture of succinate + glutamate, the respiratory rates in all metabolic states were increased dramatically.

For the sake of better understanding the following experimental results and discussion, let us consider more closely the metabolic states of RHM presented in figures 1A and 1B, as changes in respiration rates and membrane potential measured with the TPP^+-sensitive electrode. TPP^+ is a hydrophobic cation, and when RHM are added to the incubation medium containing TPP^+, mitochondria begin consume the cation because it freely crosses the inner membrane into matrix towards the negative charges located at the inner surface of the membrane. Although the exact volume of the matrix is unknown, and thus we cannot calculate the transmembrane membrane potential (ΔΨ) in mV, we can qualitatively follow the dynamics of changes and the relative values of ΔΨ by comparing experiments with the same mitochondria. The comparison of figures 1A and 1B illustrates that simultaneous measurements of O_2 consumption and ΔΨ helps to interpret data much better if something goes wrong with respiration (see Figure 1A).

In experiments with succinate + rotenone, upon addition of RHM to the incubation chamber, the maximum of ΔΨ is usually achieved in just few seconds (not shown), whereas in figure 1A and 1B it took more than 2 minutes to reach the steady-state level of ΔΨ. This is because rotenone blocks the so-called reverse electron transport (RET), which in energized mitochondria normally controls the redox state of the NADH/NAD+ system and through the activity of mitochondrial transhydrogenase directs the excessive reducing equivalents of NADH for reduction of the cytosolic $NADP^+$. NADPH is another form of chemical energy alternative to ATP. In addition, the energy-dependent RET stimulates production of superoxide radicals ($O_2^{\bullet-}$). For this reason, the increased rates of resting respiration (State 4) shown in figures 1A, 1B and 3A, 3B and 3C, evidence not the uncoupling due to increased permeability for protons, but the increased rates of $O_2^{\bullet-}$ production due to RET. Because RET is highly important mitochondrial function in a cell, in order to understand normal energy metabolism in the heart, we must not use the canonical mixture of succinate + rotenone as a substrate. The significance of the intrinsic inhibition of SDH for the heart and brain mitochondria was described recently in [20].

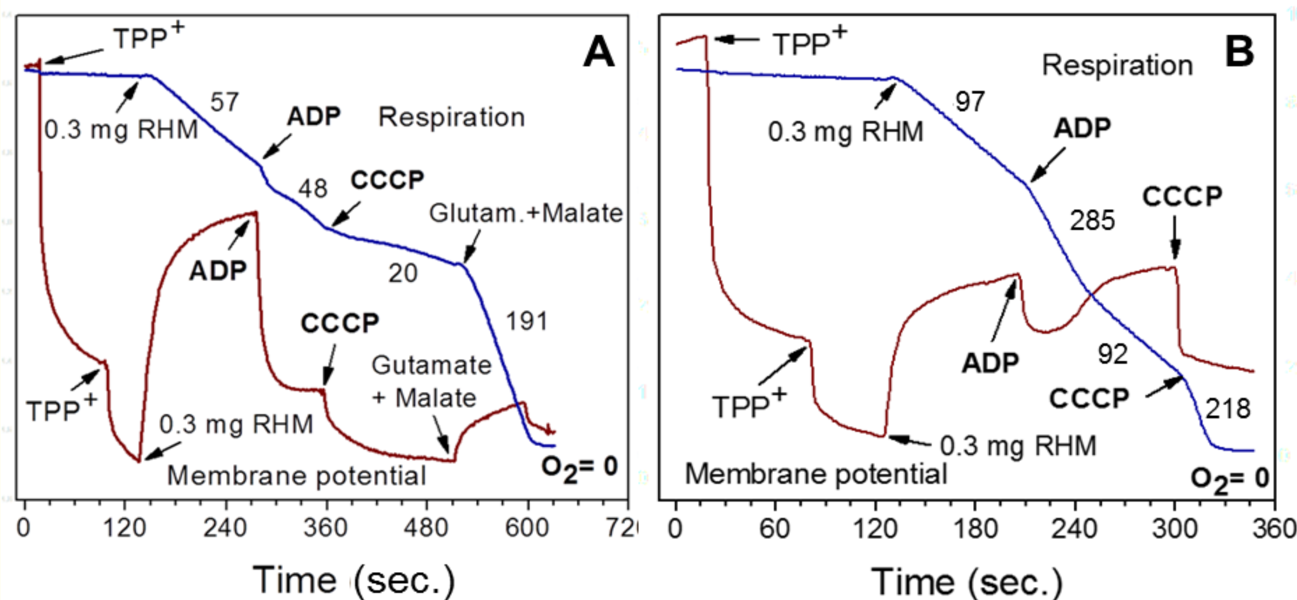

Figure 1: *Oxidation of succinate alone and in the presence of glutamate by isolated heart mitochondria from Sprague Dawley rat.*

Figure 1A: *RHM oxidizing succinate in the absence of rotenone; Figure 1B: RHM oxidizing succinate + glutamate + malate. The details of the experimental conditions are described in Methods. Substrate: succinate 5 mM, no rotenone. Titration with TPP+ was performed before addition of RHM. Additions: RHM 0.3 mg, ADP 150 μM, CCCP 0.1 μM. Membrane potential was measured with a TPP+-sensitive electrode as described in [33]. The 1st and the 2nd additions of TPP+ were 1 μM, final [TPP+] was 2 μM. Numbers at the respiration trace indicate the rates of O_2 consumption in nmol O_2/min/mg mitochondrial protein.*

Respiratory activities of RHM with various substrates added separately

Figures 2 A-F and 3 A-C show results obtained with the same batch of heart mitochondria isolated from two Sprague Dawley rats. The purpose of this large experiment was to minimize possible variations due to individual phenotypic differences in mitochondrial metabolism.

Figures 2A and 2B show correspondingly the rates of O2 consumption in the three major metabolic states by RHM oxidizing pyruvate + malate and glutamate + malate. Figure 2C shows experiment, when RHM oxidized the mixture glutamate + pyruvate + malate. One can see that there was no large difference between the three experiments. The lack of activation of oxidative phosphorylation (State 3) during simultaneous oxidation of glutamate + pyruvate by RHM is a specific distinction from the brain mitochondria, where combination of the two substrates caused a significant activation of oxidative phosphorylation [24]. Figure 2D illustrates once more that RHM do not oxidize normally succinate in the absence of rotenone due to the intrinsic inhibition of SDH. Figure 2D shows that pyruvate + malate can also release the intrinsic inhibition of SDH similar to glutamate (see Figure 1A). Figure 2E illustrates that the combination of succinate + glutamate + malate is highly effective substrate mixture for the heart mitochondria with 70% increase in the rate of oxidative phosphorylation as compared with glutamate + malate (Figure 2B).

Figure 2F shows that the natural fatty acid substrate for RHM palmitoyl-carnitine, when added alone, is a poor substrate for energization of mitochondria. This, probably, was the major reason why palmitoyl-carnitine was rarely used by researchers as a substrate for energization of the heart mitochondria. We found also that the presence of malate has no influence on oxidation of palmitoyl-carnitine.

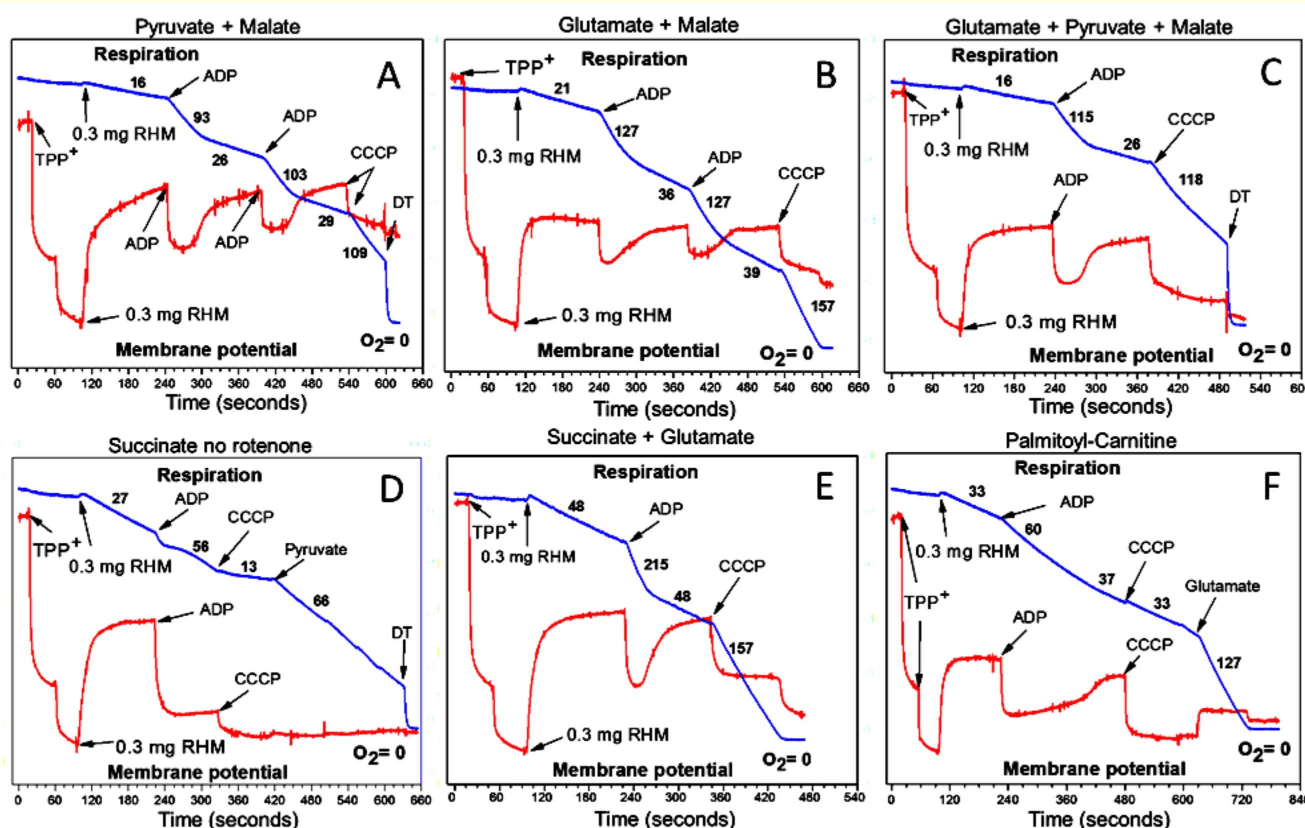

Figure 2: *Oxidation by rat heart mitochondria of major substrates: A. pyruvate + malate, B. glutamate + malate, C. glutamate + pyruvate + malate, D. succinate, no rotenone, E. Succinate + glutamate, F. palmitoyl-carnitine. Incubation conditions and additions as described in figure.*

Synergistic interactions of palmitoyl-carnitine with other mitochondrial substrates

The results presented in figures 2D and 2F show that when used separately, two natural mitochondrial metabolites succinate and palmitoyl-carnitine look like "bad" substrates for the heart mitochondria, unlike glutamate and pyruvate, which for decades served as "classical" mitochondrial substrates as well as succinate + rotenone. However, when these two "bad" substrates were mixed together, the situation was quite different (see Figure 3A). Figures 3B and 3C show that with palmitoyl-carnitine combined with pyruvate or glutamate the rates of ATP formation almost doubled, and oxygen consumption in State 4 and State 3U were also increased significantly.

Production of ROS by rat heart mitochondria during synergistic oxidation of palmitoyl-carnitine and other mitochondrial substrates

The fact that increased rates of oxygen consumption during resting respiration can be associated not with uncoupling, but rather with the increased rates of superoxide anion production, we observed first in our experiments with the rat brain mitochondria oxidizing pyruvate + glutamate substrate mixture [24]. With the heart mitochondria, simultaneous oxidation of palmitoyl-carnitine with either pyruvate, glutamate or succinate also resulted in several fold increase of the State 4 respiration as compared with the corresponding respiration rates with single substrates (compare figures 2A-F and 3A-C, see also figure 5).

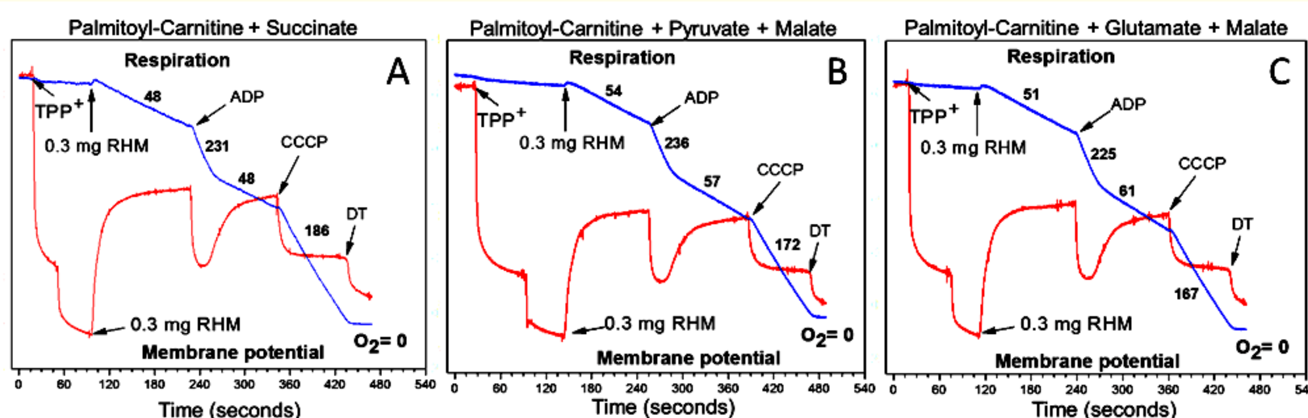

Figure 3: *Oxidation by rat heart mitochondria of palmitoyl-carnitine in the presence of: A. pyruvate + malate, B. glutamate + malate, C. succinate.*

Incubation conditions and additions as in figure 1.

Figures 4A, 4B, and 4C show the rates of ROS production as picomol H_2O_2 per minute per mg RHM, oxidizing correspondingly pyruvate, glutamate and succinate alone and as mixtures with palmitoyl carnitine. In all experiments, oxidation of the mixture of palmitoyl-carnitine with either pyruvate, glutamate or succinate dramatically increased production of superoxide radicals.

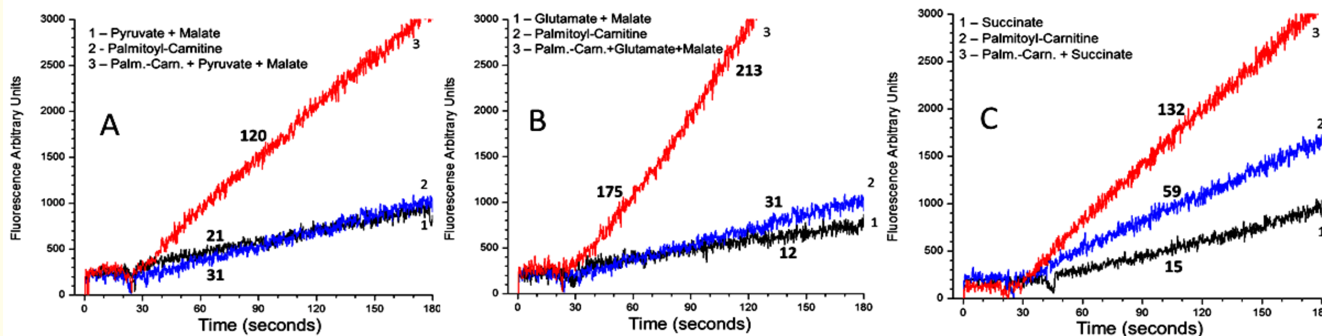

Figure 4: *Production of superoxide radicals by RHM oxidizing: 1. corresponding substrate only; 2. palmitoyl-carnitine only, and 3. palmitoyl-carnitine + substrate. Substrates: Figure 4A - pyruvate + malate, Figure 4B - glutamate + malate, and Figure 4C - succinate. Experimental conditions are described in Methods. 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 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.*

Figure 5 presents a summary of several experiments on respiratory activities and ROS production by RHM oxidizing different substrates and substrate mixtures. From these data we can conclude that the simultaneous oxidation of fatty acid with one of the alternative mitochondrial substrates pyruvate, succinate and glutamate results in: 1) significant acceleration of oxidative phosphorylation; 2) significant increase in the resting respiration; and 3) dramatic increase in production of ROS.

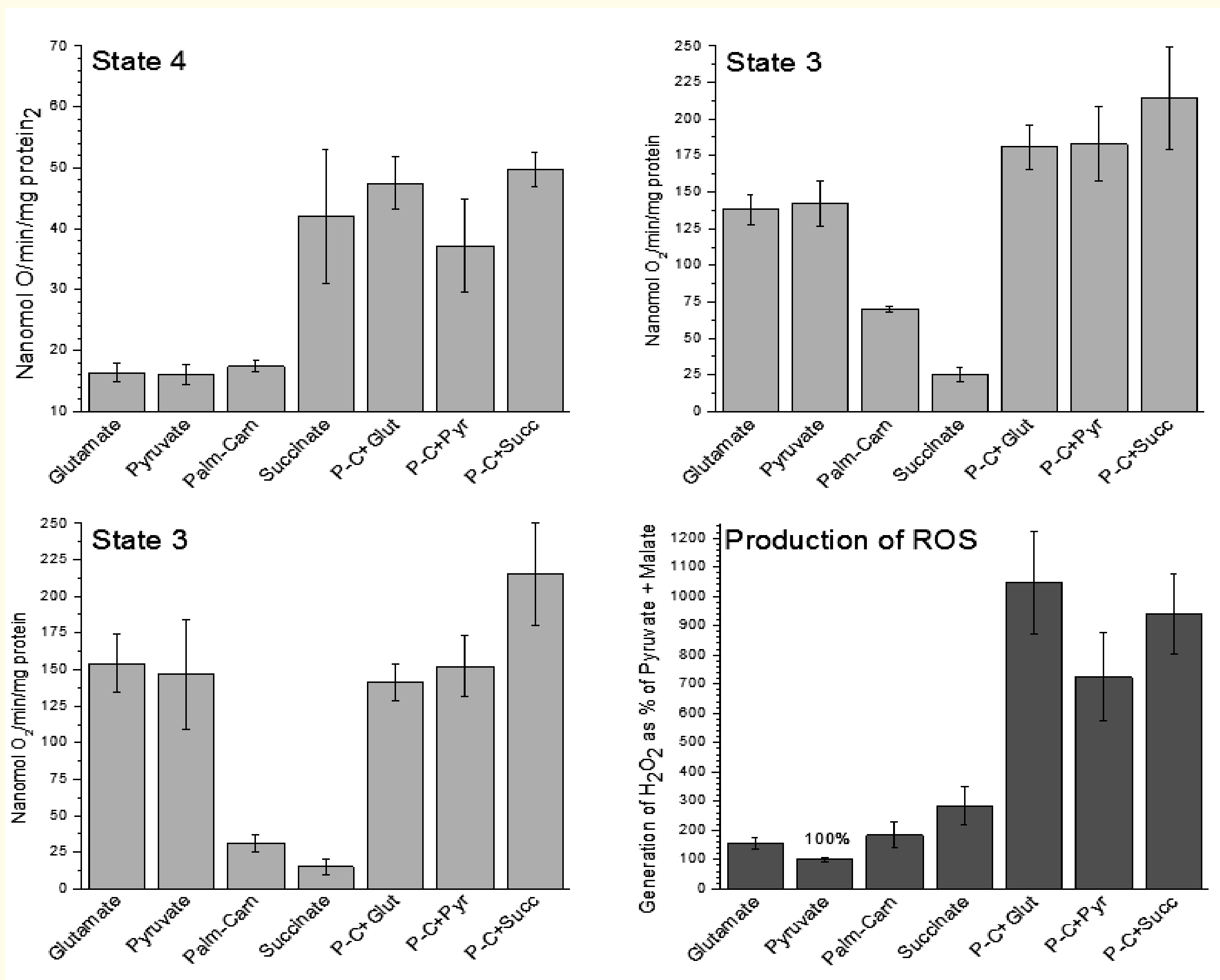

Figure 5: *Respiratory activities of RHM and ROS production during synergistic oxidations of palmitoyl-carnitine and other mitochondrial metabolites.*

Respiratory data presented as M ± SE of 4-8 separate isolations. ROS production are presented as percent of H_2O_2 production by RHM oxidizing pyruvate + malate, which was taken as 100%, as M ± SE of 3-5 experiments.

In order to understand the consequences of the simultaneous synergistic oxidation of fatty acids and other mitochondrial substrates for the heart metabolism and functioning, we have to discuss some more general aspects of energy metabolism in the body and heart.

Source of Energy (Caloric value- kcal/g)	Storage amount (time of consumption)
Carbohydrates:	
Blood glucose and	4 - 5 grams (20-30 min)
Glycogen (CV = 3.81)	100 - 120 gram (1-3 hrs.)
Amino acids (CV = 3.12)	Released during catabolism of food, damaged tissue proteins and anaplerotic reactions. The content is highly dynamic.
Acyl Fatty Acids (CV = 9.3)	Fat (Kilograms) days

Table 1: *Relative Caloric Values and Storage Amounts of major Sources of Mitochondrial Substrates.*

General Discussion

Energy stores and the timeline of substrate utilization in the heart. Although glucose, glutamate and pyruvate served as good substrates for mitochondria in experiments *in vitro*, they are not the physiologically relevant main substrates for the heart. This is because in the body the reserves for glucose are limited and much less so for amino acids. A person, who had a good supper and slept soundly over the night, in the morning has full reserves of carbohydrates in the form of blood glucose and glycogen in the liver and muscles, some amount of aminoacids in the blood, and fats as a fat tissue and free fatty acids bound in the blood to albumin and transport proteins. How long each of these storages will last as the source of hydrogen in a person who performs a mild work, say, a quiet walk? And what organs will be consuming these stores?

Let us first consider carbohydrates. The content of glucose in the blood is close to 1000 mg per 1L that is 5 grams in an average person with 5 liters of blood. This amount of glucose will be consumed by erythrocytes alone just in about 20 minutes (A. Panov, unpublished data), and in a much less time, if glucose is also consumed by the brain, spinal cord, heart, skeletal muscles, kidneys and liver. Usually, the largest storage of glucose in the liver as glycogen is about 100 - 120 gram [26]. Only glucose from the liver's glycogen can be shared with other organs [26]. We should also keep in mind that fat tissue also consumes large amount of glucose. This is because in the fat cells there is a constant cycling of fatty acids: first triglycerides split into free fatty acids and glycerol, and then the same fatty acids become reconnected to a new molecule of glycerol formed from glucose, to generate a triglyceride, while the "old" glycerol is catabolized.

As a result, after about 2 - 3 hours of mild work, all reserves of carbohydrates (glucose) will be depleted. Therefore, in order to maintain the physiologically steady level of glucose in the blood, the liver metabolism must switch early from glycolysis to gluconeogenesis. The constant level of glucose in the blood even after prolonged starvation is maintained for the sake of normal functioning of cells, which have absolute requirement for glucose: erythrocytes, nerve tissue, bone marrow and fat tissue. The storage of amino acids is very small, and during gluconeogenesis and neuronal activity the pool of amino acids must be constantly replenished by anaplerotic transamination of α-ketoglutarate, pyruvate, or proteolysis of proteins. Fat is the largest and most energetically rich storage of hydrogen. Thus, the incessantly working organs, such as heart and kidneys, must utilize fatty acids practically at all times as the major source of hydrogen [18].

The experiments presented in this paper suggest that effective oxidation of fatty acids requires constant consumption of other metabolites derived from either glucose or amino acids. This explains the essence of the old saying that "the heart burns fatty acids in the flame of carbohydrates", and we can add: ..."and amino acids".

At this point, we cannot give a reasonable explanation to the mechanisms of synergistic interactions between fatty acids and other structurally different metabolites during their oxidation by mitochondria. We also do not know how much of alternative substrates is necessary to facilitate oxidations of fatty acids. Our experience with the brain and spinal cord mitochondria suggest that in different organs there are different optimal substrates combinations which are specific for each organ in accord with the organ's functions and metabo-

lism [24,27]. It is now becoming more and more evident that fatty acids, as the source of hydrogen, are more indispensable than thought before, even for the nerve tissue. Earlier, we have suggested that fatty acids in the nerves provide energy for anaplerotic replenishment of glutamine and glutamate [27]. We have also shown that non-synaptic neuronal mitochondria oxidize palmitoyl-carnitine perfectly well in the presence of succinate, pyruvate and glutamate with similar enhancement in the rates of ATP and ROS production [27], as we show in this paper for the RHM. This raises the problem of the oxidative stress in aged individuals who have the metabolic syndrome.

Metabolic syndrome as a prerequisite for CVD: Metabolic syndrome develops with age in men relatively gradually [28], whereas in women the transition may occur fast after menopause [29]. People with metabolic syndrome undergo both structural alterations in the body and changes in the metabolic patterns including: 1) increased central (intra-abdominal) body fat; 2) a shift toward a more atherogenic lipid profile, with increased low density lipoprotein and triglycerides levels, reduced high density lipoprotein, and small, dense low density lipoprotein particles; and 3) increased glucose and insulin levels [29]. The metabolic syndrome represents a cluster of abnormalities, including obesity, insulin resistance, dyslipidemia and Type 2 diabetes that increases the risk of developing cardiovascular diseases, such as coronary artery disease and heart failure [28-30]. Although there is enormous amount of literature on metabolic syndrome and the associated diseases, our analysis of literature suggests that there are some misconceptions regarding the essence of the metabolic syndrome and metabolic roles of fatty acids. Of most importance is the question: how and why the metabolic syndrome develops. Obesity at younger age is not fully equivalent to the metabolic syndrome.

During ontogenesis, every stage of our life is controlled by different set of stage-specific genes. We suggest that at old age, when the metabolic syndrome develops, our body becomes controlled by evolutionary more ancient genes, for which there was no natural selection. These ancient genes that we inherited from our Ice Age ancestors, support metabolic features and the body structure similar to those, which can be observed among the current indigenous populations of the Polar Regions. In other words, development of metabolic syndrome is not a consequence of dysregulation of metabolism due to oxidative stress, wrong diet or other reasons, but represent a completely new set of the stage-specific genes. This suggestion might explain manifestation of important features of the metabolic syndrome, which was named “metabolic inflexibility” as a key dysfunction of the cluster of disease states encompassed by the term “metabolic syndrome” [28,29].

Several authors suggested that the manipulation of myocardial substrate oxidation toward greater carbohydrate oxidation and less fatty acids oxidation may improve ventricular performance and slow the progression of heart dysfunction [18,30-33]. We believe that during this type of research we have to keep in mind the following: 1. There exist large genetic variations in the heart energy metabolism and antioxidative stress defenses [20]; 2. During experiments, the responses of younger animals may be different from those in people with the “metabolic inflexibility”; and finally 3. During heart failure in patients with metabolic syndrome, the symptoms may result from insufficient supply of supporting synergism metabolites derived from glucose or amino acids.

Role of synergistic oxidation of fatty acids in oxidative stress: Increased production of ROS during synergistic oxidation of fatty acids and “supportive” metabolites, evidently results in accelerated aging of the heart by mechanism, which involves activation of the isoprostane pathway of lipid peroxidation by perhydroxyl radical [34-36]. But a thorough discussion of this subject is beyond the main scope of this paper.

Conclusion

For the first time we provide data that at the mitochondrial level there is no competition for oxidation between glucose product pyruvate, but a synergistic enhancement of oxidation of fatty acids in the presence of either pyruvate, succinate or glutamate, which we designate as “supportive metabolites”. The synergistic oxidation of palmitoyl-carnitine together with supportive metabolites results in significant enhancement of ATP production and state 4 respiration. The latter is caused by enhancement of the superoxide radical production, which serve as a sink for electrons due to enhanced reversed electron transport. This suggest that in general enhancement of ROS production in resting heart results in accelerated aging due to activation of the isoprostane lipid peroxidation.

Conflict of Interest

There is no conflict of interest between the author and the research Institute where the author is affiliated.

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