L-Carnitine and α-Lipoic Acid Improve Age-Associated Decline in Mitochondrial Respiratory Chain Activity of Rat Heart Muscle

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The aging process is characterized by a general decline in physiological functions that affects many tissues and increases the risk of death. In the present investigation using various substrates, the respiration rate was observed in young, middle-aged, and aged rats upon administration of carnitine (300 mg/kg body weight) and lipoic acid (100 mg/kg body weight). We observed that the rate of respiration, both State 3 and respiratory control ratio, decreased significantly in aged rats after using various substrates (except succinate). An increase in the State 4 respiration was observed in aged rats when β-hydroxybutyrate as well as pyruvate and malate were used as substrates, whereas no change in the adenosine diphosphate/oxygen ratio was observed. These changes were brought to normal levels upon cosupplementation of carnitine and lipoic acid. Thus, this study provides evidence for the role of carnitine and lipoic acid in alleviating the age-related decline in mitochondrial respiratory activity.

OXYGEN is used by the mitochondria in cells to produce the high-energy compound, adenosine triphosphate (ATP). ATP is used as an energy source for virtually every species of life on earth. The energy status of cells, tissues, organs, and the whole body can be defined by the ability to produce and maintain threshold levels of ATP. The decline in ATP production is a significant impairment in old age (1).

Harman (2) was among the first investigators to propose that mitochondria may have a central role in the process of aging. Mitochondria and mitochondrial DNA (mtDNA) are essential for survival at the cellular level, yet organisms are particularly vulnerable to damage. Their high consumption of metabolic fuels in the pursuit of ATP production via the inner membrane–incorporated respiratory chain releases the toxic by-products known as free radicals (3,4). These highly unstable molecules, which are normally disposed of by free radical scavenging enzymes can escape these defense mechanisms and increase the levels of oxidative stress. During aging, some of the free radical scavenging systems are decreased (5,6), so that more free radicals escape, increasing the level of oxidative stress within the organelle. Thus according to the “mitochondrial theory of aging,” the enhanced production of reactive oxygen species and accumulation of mtDNA mutations in mitochondria of postmitotic cells are contributory factors to age-related deterioration (7).

MtDNA with oxidative modification and/or mutation are transcribed and translated to produce defective protein subunits that are assembled to form defective electron transport chain proteins, especially in complexes I and IV (8). The impaired electron transport chain not only works less efficiently in ATP synthesis (9), but it also generates more reactive oxygen species, which further enhances oxidative damage to various biomolecules in mitochondria (10).

During aging, nutrient consumption, absorption, or utilization is decreased, hence therapeutic nutrient supplementation is necessary. L-Carnitine is a conditionally essential nutrient synthesized from the amino acids lysine and methionine in the human liver, brain, and kidney, and it functions in the transport of activated fatty acids over the mitochondrial inner membrane in eukaryotes. It is indispensable for the β-oxidation by mitochondria of long-chain fatty acids. Thus, it has key functions in substrate flux and energy production (11). In this context it has been shown that L-carnitine improves the transcription of mtDNA in senescent rats probably by increasing oxygen consumption and therefore ATP synthesis (12).

α-Lipoic acid is a thiol present in all eukaryotic and prokaryotic cells, in which it plays an important role in different metabolic pathways (13). It is found naturally in mitochondria as the coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Furthermore, lipoic acid inhibits age-related alterations in the expression of genes involved in the extracellular matrix, cellular structure, and protein turnover, and it lowers the expression of genes encoding major histocompatibility complex components (14) evidencing that supplementation with lipoic acid results in transcriptional alterations consistent with a state of reduced oxidative stress in the heart. Previous studies in our laboratory have shown that the combined effect of carnitine and lipoic acid improves the antioxidant status and increases the activities of electron transport complexes (6,15).

The purpose of the present study is to investigate the respiration rates of mitochondria isolated from heart tissue of young and aged rats and evaluate whether dietary...
supplementation of carnitine and lipoic acid could alleviate these age-associated alterations in mitochondria.

**METHODS**

**Materials**

L-Carnitine and DL-\(\alpha\)-lipoic acid were purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals were of reagent grade and were obtained from Glaxo Laboratories, CDH division (Mumbai, India) and Sarabhai. M. Chemicals (Baroda, India).

**Animals and Drug Treatment**

Male albino rats of Wistar strain approximately 3–4 months old (young), 13–15 months old (middle aged), and more than 24 months old (aged) were used in this study. The animals were housed in large spacious cages and given food and water ad libitum. The cages were well ventilated, and the animals were on a 12-hour light/dark cycle throughout the experimental period. This study was performed in accordance with the guidelines for animal experimentation of the University of Madras, and animal ethical clearance was obtained.

The animals were divided into 6 groups, each consisting of six animals: Group I (control young rats); Group II (young rats administered carnitine and lipoic acid); Group III (control middle-aged rats); Group IV (middle-aged rats administered carnitine and lipoic acid); Group V (control aged rats); and Group VI (aged rats administered carnitine and lipoic acid).

L-Carnitine (300 mg/kg body weight [bw]/day; 16) was dissolved in 0.89% physiological saline and DL-\(\alpha\)-lipoic acid (100 mg/kg bw/day; 17) was dissolved in 0.5% KOH in physiological saline and administered orally using an intragastric cannula for 28 days. Control animals received physiological saline alone.

On completion of the experimental period, animals were killed by cervical decapitation. Heart was excised immediately, immersed in physiological saline, and used for further analysis.

**Isolation of Mitochondria**

Heart mitochondria were isolated by a modification of the method of Chance and Hagihara (18). The heart was removed, weighed, and then chilled in ice-cold Buffer A (0.32 M sucrose, 10 mM EDTA, and 5 mM Tris, pH 7.3). The heart tissue was suspended in Buffer A (10 ml/g of heart tissue), which contained collagenase at 0.5 mg/ml, and was digested by incubation of this suspension for 10–12 minutes at 4°C. The digested tissue was then homogenized at 4°C. The homogenate was diluted with an equal volume of Buffer A containing bovine serum albumin at 1 mg/ml, and the digestion was continued for an additional 15–20 minutes at 4°C. The pellet was homogenized gently a second time, and the homogenate was centrifuged at 300 g for 3 minutes. The pellet was discarded, and the supernatant was centrifuged at 500 g for 5 minutes. The resulting supernatant was then centrifuged at 8000 g for 10 minutes, and the pellet, containing mitochondria, was washed twice with Buffer A. The mitochondria were resuspended in Buffer A (2 ml/g of heart tissue) and were maintained at 4°C until used for the experiments.

Purity of the mitochondria was checked and normalized using the specific marker enzyme, succinate dehydrogenase. The activity of succinate dehydrogenase was assayed by the method of Slater and Bonner (19), in which the rate of reduction of potassium ferricyanide to potassium ferrocyanide by succinate was measured in the assay medium containing phosphate buffer (pH 7.6), EDTA, and potassium cyanide. The change in optical density was recorded at 15-second intervals for 5 minutes at 420 nm using a Shimadzu spectrophotometer (model UV-1700; Shimadzu Pvt Ltd, Singapore).

**Mitochondrial Respiration Measurements**

Oxygen consumption was measured polarographically in isolated mitochondria by using a Clark-type oxygen electrode and an oxygen monitor (model 55 oxygen monitor; Yellow Springs Instruments, Yellow Springs, OH) at 26°C (20). For calibration, the oxygen content of an air-saturated respiration buffer was assumed to be 406 nmoles O2/ml (21) under the conditions of the experiment. An aliquot of the assay medium (0.3 M sucrose, 10 mM potassium phosphate, 1 mM EDTA, and bovine serum albumin at 1 mg/ml; pH 7.5) was added to the reaction vessel and was allowed to equilibrate for several minutes at 26°C, then 0.2 ml of the mitochondrial suspension, which contained 2–5 mg of protein, was added to the assay medium. After a short equilibration period, the oxygen electrode was inserted into the reaction vessel, and the substrates were added at the following concentrations: 4 mM \(\beta\)-hydroxybutyrate, 4 mM succinate, 4 mM glutamate, 4 mM pyruvate, and 4 mM malate. State 4 respiration was determined immediately after each substrate was added to the reaction vessel. State 3 respiration was initiated by the addition of 10 µl of 0.25 mM adenosine diphosphate to the assay medium. The respiratory control ratio (ratio between State 3 and State 4) and the adenosine diphosphate/oxygen ratios were calculated as described (20). The mitochondrial protein concentration was determined as described by Lowry and colleagues (22) using bovine serum albumin as the standard. The mitochondrial respiration was expressed as nanomoles of oxygen utilized per minute per milligram of protein.

**Statistical Significance**

Values were measured in duplicate and expressed as mean ± standard deviation for six rats in each group. Statistical significance of changes in different groups was evaluated by one-way analysis of variance (ANOVA) followed by Duncan’s post hoc test using the SPSS package (version 7.5; SPSS, Chicago, IL). Differences were considered statistically significant at values of \(p < .05\).

**RESULTS**

The respiratory activity of mitochondria from rat heart was assayed polarographically, within 1 hour of isolation, by using succinate, hydroxybutyrate, glutamate, malate, and pyruvate as substrates. ADP was also added to the same
sample to monitor the State 3 and respiratory control ratio. Figure 1 shows the representative polarograph of young and aged mitochondria.

The respiratory rates measured in State 3 with all the substrates are plotted in Figure 2A as a function of age. Statistical analyses of the data, carried out by plotting the specific arithmetic values of respiratory rates as a function of age, showed a significant negative correlation between the respiratory rates measured and all the substrates, except succinate. Table 1 shows the State 3 rates as normalized with mitochondrial protein and with succinate dehydrogenase activity. There was no significant change in the State 3 respiration in both middle-aged and aged rats when using succinate as substrate. The State 3 respiration was found to decrease significantly ($p < .005$) in both Group III and Group V rats when using the various other substrates. The decrease was 24% and 38% in middle-aged and aged rats, respectively, when using β-hydroxybutyrate as substrate, 15% and 30% when using glutamate, 22% and 34% when using glutamate and malate, and 14% and 29% when using pyruvate and malate as substrates, respectively. The State 3 respiration rates returned to normalcy upon administration of carnitine and lipoic acid.

Age affected the State 4 respiration (Figure 2) only to a certain extent. The respiration rate did not change significantly upon using succinate, glutamate, or glutamate and malate as substrates in both middle-aged and aged rats, but the respiration increased (Table 2) by 1.15-fold in aged rats when β-hydroxybutyrate as well as pyruvate and malate were used.

Figure 2 depicts the statistical analysis of respiratory control ratio in control and treated rats with age. A significant negative correlation was observed with correlation coefficients significant at $p < .01$. As with States 3 and 4, there was no change in the respiration when succinate was used as the substrate (Table 3). However, the respiratory control ratio decreased significantly ($p < .05$) with the other substrates used. The decrease was 31% and 47%, respectively, in middle-aged and aged rats when using β-hydroxybutyrate as substrate, 24% and 36% when using glutamate, 24% and 38% when using pyruvate and malate, and 23% and 39% when using pyruvate and malate as substrates, respectively. Supplementation with carnitine and lipoic acid brought these levels to normalcy. No significant change was observed in young rats treated with carnitine and lipoic acid. The efficacy of the drug was more pronounced in combined treatment of carnitine and lipoic acid than when treated alone. Table 4 presents the ADP/O ratio of control and treated rats. The ADP/O ratio did not change as a function of age when using any of the substrates.

**DISCUSSION**

The decline in the ability of cells to produce energy in the form of ATP is one popular explanation for the age-related decline in the physiological function of an organism. Therefore, many investigators have studied the effect of senescence on mitochondrial respiratory function. Considerable evidence has accumulated that a failure of mitochondrial energy generation may be important in the aging process.

The results from our studies with heart mitochondria showed that when the rate of respiration was highest (State 3), a sharp decrease in mitochondrial function was observed with increasing age. A similar decrease in the efficiency of State 3 mitochondrial respiration has been demonstrated in intact mitochondria isolated from human skeletal muscle with rates being about half those of younger muscles (23). Our observation that the rate of State 3 respiration decreased during senescence is in line with a recent report (24). Group IV and Group VI rats showed improvement in the levels of State 3 respiration when compared to their middle-aged and aged counterparts.

State 4 respiration is characterized by no ATP production, a backed-up electron transport chain, and substantially reduced oxygen consumption. Oxygen consumption in State 4 respiration is generally assumed to reflect the summed leak processes across the inner mitochondrial membrane. We observed no significant change in the levels of State 4 respiration in middle-aged rats, but the rate increased in aged rats when β-hydroxybutyrate as well as pyruvate and malate were used as substrates, indicating an increased proton leak during aging. It has been shown that State 4 respiration increases significantly in damaged mitochondria (25). It is interesting that treatment with carnitine and lipoic acid decreased these levels significantly. It can be suggested that carnitine and lipoic acid increase the electron flow through the electron transport chain; this increase may decrease proton leak in aged rats.

In the present study we observed that the ADP/O ratios were unaltered when using all the substrates in both middle-aged as well as aged rats. There was also no significant change in the respiratory control ratios of succinate-supported respiration of the heart mitochondria during aging. However, the rate was found to decrease significantly with age when the other substrates were used. This decrease
might be due to loss of mitochondrial membrane integrity and decreased activities of electron transport chain complexes (26). The loss of mitochondrial membrane integrity leads to impaired proton gradient, subsequently decreasing the synthesis of ATP by ATP synthase. Therefore, the research provides evidence that the structural integrity of mitochondrial membranes gradually decayed during the aging process, indicating that the basal level respiratory activities also declined with age. However, on treatment with carnitine and lipoic acid, the integrity of the mitochondria was retained. Earlier reports from our laboratory show that carnitine and lipoic acid have the potential to

Figure 2. A, Statistical analysis of relationship between State 3 respiration of heart mitochondria and age. Significances were measured at $p < .05$ ($n = 36$). For experimental details, see the Methods section. B, Statistical analysis of relationship between State 4 respiration of heart mitochondria and age. No statistical significance was observed at $p < .05$ ($n = 36$). C, Statistical analysis of relationship between respiratory control ratio of heart mitochondria and age ($n = 36$). A negative correlation was observed using various substrates except succinate, which did not show any significance. Significances were measured at $p < .05$. D, Statistical analysis of relationship between adenosine diphosphate/oxygen ratio of heart mitochondria and age. No statistical significance was observed at $p < .05$ ($n = 36$).
bring the activities of electron transport complexes to normalcy (15).

The above results conclude that respiration rate is an age-dependent process. This result, in conjunction with evidence that oxidative damage is a major contributor to the aging process (27), leads to the proposal that oxygen free radicals, generated as by-products of mitochondrial respiration, may impair mitochondrial respiratory chain function. This impairment could be caused by damage to membrane lipids, respiratory chain proteins, or mtDNA. Even in the presence of protective antioxidant systems, damage to proteins, lipids, nucleic acids, and carbohydrates can be observed (under normal physiological conditions) to increase with age (28). Thus, we strongly suggest that this could be the reason that the senescent tissues show a significant decline in their ability to produce ATP at a very high rate. This decline in

Figure 2. Continued.
the rate of ATP formation could be critical to a senescent organism when it is exposed to severe stress and when demand is placed on various tissues for a large amount of energy. Therefore, it seems very likely that age-related changes at the mitochondrial level are important in the decline in physiological function that accompanies senescence.

Treatment with carnitine and lipoic acid reversed the age-related alterations in mitochondria. As mentioned earlier, carnitine is essential for shuttling fatty acids into the mitochondria for $\beta$ oxidation. It has been observed that the levels of carnitine and lipoic acid decrease during aging (29,30). Therefore, supplementation of these nutrients will increase the substrate availability for $\beta$ oxidation and subsequently for tricarboxylic acid cycle. Thus on the whole the increase in the respiration rates by carnitine could be by increasing the substrates for mitochondrial respiration.
Carnitine, in contrast, also has been shown to improve the level of cardiolipin, a mitochondrial phospholipid that maintains the mitochondrial structure from oxidative damage, thereby protecting the membrane against damage (31). Alternatively, the increase in energy metabolism could serve to reestablish correct ionic conditions in the mitochondria and, in general, to reduce membrane damage induced by aging (12).

The decrease in the respiration rates is also due to the increased free radical generation in the senescent mitochondria. Lipoic acid, by serving both as an antioxidant and as a cofactor of the mitochondrial enzymes, could have
Table 1. Effect of Carnitine and Lipoic Acid on the State 3 Respiration in Heart Mitochondria of Control and Treated Animals

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Young Controls</th>
<th>Young Treated</th>
<th>Middle-Aged Controls</th>
<th>Middle-Aged Treated</th>
<th>Aged Controls</th>
<th>Aged Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
</tr>
<tr>
<td>Succinate</td>
<td>79.86 ± 9.07</td>
<td>14.16 ± 1.56</td>
<td>83.38 ± 9.35</td>
<td>14.23 ± 1.56</td>
<td>74.80 ± 8.51</td>
<td>14.99 ± 1.66</td>
</tr>
<tr>
<td>β HB</td>
<td>16.20 ± 1.85</td>
<td>3.06 ± 0.34</td>
<td>16.78 ± 1.94</td>
<td>3.18 ± 0.35</td>
<td>12.30 ± 1.32</td>
<td>2.33 ± 0.25</td>
</tr>
<tr>
<td>Glutamate</td>
<td>33.00 ± 3.79</td>
<td>6.25 ± 0.69</td>
<td>33.20 ± 3.79</td>
<td>6.28 ± 0.69</td>
<td>27.97 ± 3.22</td>
<td>5.29 ± 0.59</td>
</tr>
<tr>
<td>Glu + Mal</td>
<td>52.20 ± 5.96</td>
<td>9.88 ± 1.09</td>
<td>53.60 ± 6.13</td>
<td>10.14 ± 1.12</td>
<td>40.71 ± 4.81</td>
<td>7.69 ± 0.82</td>
</tr>
<tr>
<td>Mal + Pyr</td>
<td>23.20 ± 2.64</td>
<td>4.39 ± 0.48</td>
<td>23.48 ± 2.67</td>
<td>4.44 ± 0.49</td>
<td>19.83 ± 1.91</td>
<td>3.76 ± 0.39</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± standard deviation for six rats in each group. Respiration rate normalized to mitochondrial (mt) protein was expressed as nanomoles of oxygen utilized per minute per milligram of protein. Respiration rate normalized to succinate dehydrogenase (SDH) was expressed as nanomoles of oxygen utilized per minute per SDH activity.

* Differences compared with young control rats, p < .05.
† Differences compared with middle-aged control rats, p < .05.
‡ Differences compared with aged control rats, p < .0.5.

HB = hydroxybutyrate; Glu = glutamine; Mal = malate; Pyr = pyruvate.

Table 2. Effect of Carnitine and Lipoic Acid on the State 4 Respiration of Heart Mitochondria in Control and Treated Animals

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Young Control</th>
<th>Young Treated</th>
<th>Middle-Aged Control</th>
<th>Middle-Aged Treated</th>
<th>Aged Control</th>
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<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
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<tr>
<td>Succinate</td>
<td>12.1 ± 1.37</td>
<td>2.29 ± 0.24</td>
<td>12.15 ± 1.37</td>
<td>2.29 ± 0.24</td>
<td>12.9 ± 1.37</td>
<td>2.44 ± 0.25</td>
</tr>
<tr>
<td>β HB</td>
<td>5.06 ± 0.58</td>
<td>0.96 ± 0.01</td>
<td>5.1 ± 0.58</td>
<td>0.97 ± 0.1</td>
<td>5.59 ± 0.63</td>
<td>0.70 ± 0.11</td>
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<tr>
<td>Glutamate</td>
<td>3.7 ± 0.41</td>
<td>0.7 ± 0.07</td>
<td>3.64 ± 0.41</td>
<td>0.69 ± 0.07</td>
<td>4.16 ± 0.47</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td>Glu + Mal</td>
<td>10.07 ± 1.15</td>
<td>19 ± 0.2</td>
<td>10.23 ± 1.18</td>
<td>19.3 ± 0.21</td>
<td>10.4 ± 0.13</td>
<td>1.96 ± 0.2</td>
</tr>
<tr>
<td>Mal + Pyr</td>
<td>3.9 ± 0.44</td>
<td>0.74 ± 0.08</td>
<td>3.89 ± 0.48</td>
<td>0.73 ± 0.08</td>
<td>4.4 ± 0.5</td>
<td>0.83 ± 0.09</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± standard deviation for six rats in each group. Respiration rate normalized to mitochondrial (mt) protein was expressed as nanomoles of oxygen utilized per minute per milligram of protein. Respiration rate normalized to succinate dehydrogenase (SDH) was expressed as nanomoles of oxygen utilized per minute per SDH activity.

* Differences compared with young control rats, p < .05.
HB = hydroxybutyrate; Glu = glutamate; Mal = malate; Pyr = pyruvate.
### Table 3. Effect of Carnitine and Lipoic Acid on the Respiratory Control Ratio of Heart Mitochondria in Control and Treated Animals

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Young Control</th>
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<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
</tr>
<tr>
<td>Succinate</td>
<td>6.18 ± 0.69</td>
<td>1.17 ± 0.12</td>
<td>6.19 ± 0.96</td>
<td>1.17 ± 0.12</td>
<td>6.14 ± 0.69</td>
<td>1.16 ± 0.12</td>
</tr>
<tr>
<td>β HB</td>
<td>3.20 ± 0.36</td>
<td>0.6 ± 0.06</td>
<td>3.29 ± 0.35</td>
<td>0.62 ± 0.06</td>
<td>2.20 ± 0.25*</td>
<td>0.41 ± 0.04*</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8.88 ± 1.04</td>
<td>1.68 ± 0.18</td>
<td>9.11 ± 1.05</td>
<td>1.72 ± 0.18</td>
<td>6.72 ± 0.58*</td>
<td>1.27 ± 0.11*</td>
</tr>
<tr>
<td>Glu + Mal</td>
<td>5.18 ± 0.61</td>
<td>0.98 ± 0.1</td>
<td>5.23 ± 0.58</td>
<td>0.99 ± 0.1</td>
<td>3.90 ± 0.44*</td>
<td>0.74 ± 0.08*</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± standard deviation for six rats in each group. *Differences compared with young control rats, p < .05. †Differences compared with middle-aged control rats, p < .05. Mt = mitochondrial; HB = hydroxybutyrate; Glu = glutamate; Mal = malate; Pyr = pyruvate.

### Table 4. Effect of Carnitine and Lipoic Acid on the Adenosine Diphosphate: Oxygen (ADP/O) Ratio of Heart Mitochondria in Control and Treated Animals

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Young Control</th>
<th>Young Treated</th>
<th>Middle-Aged Control</th>
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<td>Normalized to SDH</td>
<td>Normalized to mt Protein</td>
<td>Normalized to SDH</td>
</tr>
<tr>
<td>Succinate</td>
<td>1.8 ± 0.2</td>
<td>0.34 ± 0.04</td>
<td>1.93 ± 0.21</td>
<td>0.34 ± 0.04</td>
<td>1.72 ± 0.20</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>β HB</td>
<td>2.89 ± 0.32</td>
<td>0.55 ± 0.06</td>
<td>2.9 ± 0.32</td>
<td>0.55 ± 0.05</td>
<td>2.83 ± 0.31</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Glutamate</td>
<td>2.82 ± 0.31</td>
<td>0.54 ± 0.06</td>
<td>2.86 ± 0.31</td>
<td>0.55 ± 0.06</td>
<td>2.75 ± 0.28</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>Glu + Mal</td>
<td>2.85 ± 0.33</td>
<td>0.54 ± 0.06</td>
<td>2.86 ± 0.31</td>
<td>0.55 ± 0.06</td>
<td>2.79 ± 0.32</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Mal + Pyr</td>
<td>2.83 ± 0.3</td>
<td>0.54 ± 0.06</td>
<td>2.84 ± 0.3</td>
<td>0.54 ± 0.06</td>
<td>2.78 ± 0.35</td>
<td>0.53 ± 0.06</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± standard deviation for six rats in each group. Mt = mitochondrial; HB = hydroxybutyrate; Glu = glutamate; Mal = malate; Pyr = pyruvate.
increased the rates of respiration in aged rats by decreasing oxidative stress. Lipoic acid has been shown to prevent mitochondrial membrane from oxidative stress by improving the antioxidant defense system (32).

Conclusion
Carnitine and lipoic acid may act synergistically to improve age-related decline in mitochondrial bioenergetics, recuperating fatty acid and glucose catabolism, thereby increasing energy production and metabolic rate and decreasing oxidative stress.

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