Voluntary physical activity alterations in endothelial nitric oxide synthase knockout mice

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Momken, Iman, Patrick Lechène, Renée Ventura-Clapier, and Vladimir Veksler. Voluntary physical activity alterations in endothelial nitric oxide synthase knockout mice. Am J Physiol Heart Circ Physiol 287: H914–H920, 2004; 10.1152/ajpheart.00651.2003.—One of the main factors that control vasoreactivity and angiogenesis is nitric oxide produced by endothelial nitric oxide synthase (eNOS). We recently showed that knocking out eNOS induces an important reduction of mitochondrial oxidative capacity in slow-twitch skeletal muscle. Here we investigated eNOS’s role in physical activity and contribution to adaptation of muscle energy metabolism to exercise conditions. Physical capacity of mice null for the eNOS isoform (eNOS−/−) was estimated for 8 wk with a voluntary wheel-running protocol. In parallel, we studied energy metabolism enzyme profiles and their response to voluntary exercise in cardiac and slow-twitch soleus (Sol) and fast-twitch gastrocnemius (Gast) skeletal muscles. Weekly averaged running distance was two times lower for eNOS−/− (4.09 ± 0.42 km/day) than for wild-type (WT; 7.74 ± 0.42 km/day; P < 0.01) mice. Average maximal speed of running was also lower in eNOS−/− (17.2 ± 1.4 m/min) than WT (21.2 ± 0.9 m/min; P < 0.01) mice. Voluntary exercise influenced adaptation to exercise specifically in Sol muscle. Physical activity significantly increased Sol weight by 22% (P < 0.05) in WT but not eNOS−/− mice. WT Sol muscle did not change its metabolic profile in response to exercise, in contrast to eNOS−/− muscle, in which physical activity decreased cytochrome-c oxidase (COX; −36%; P < 0.05), citrate synthase (−37%; P < 0.06), and creatine kinase (−24%; P < 0.01) activities. Voluntary exercise did not change energy enzyme profile in heart (except for 39% increase in COX activity in WT) or Gast muscle. These results suggest that eNOS is necessary for maintaining a suitable physical capacity and that when eNOS is downregulated, even moderate exercise could worsen energy metabolism specifically in oxidative skeletal muscle.

mice; exercise; mitochondria; energy metabolism

Many vasoreactive factors contribute to maintain adequate blood flow in muscles under different physiological conditions. One of the main factors that control vasoreactivity and angiogenesis is endothelium-derived NO (1, 14, 42). This substance is produced in a reaction catalyzed by NO synthase (NOS), transforming l-arginine to l-citrulline. Among the NOS iso-enzymes—neuronal (nNOS), inducible (iNOS), and endothelial (eNOS)—the latter is the major isoform expressed in endothelial cells throughout the vascular bed. Mice lacking eNOS show reduced angiogenic ability (41), and inhibition of NOS has been shown to inhibit vascular remodeling in rabbits (51). Indeed, we recently showed (40) that deficiency in eNOS reduces oxidative capacity in slow-twitch skeletal muscle. However, the functional consequences of such a deficiency are not known.

On the other hand, in mammals training induces an increase of vascularization in muscles that tightly correlates with mitochondrial volume density and enzymes involved in energy metabolism (21, 24, 36, 50). NO contributes to the regulation of microcirculation during exercise, but, in turn, the NOS system has been shown to adapt to training (for review, see Refs. 26 and 29). Koller et al. (32) showed that short-term daily exercise increased the dilatory response of arterioles to NO in rat skeletal muscles. Exercise also increased the expression of eNOS in coronary vessels and the release of NO in skeletal muscle vessels in rats and mice (31, 33). This adaptation might contribute to the increased functional capacity and cardio-protective effects associated with higher physical activity (for review, see Ref. 29). Moreover, training could lead to improvement of the energy transport system in muscle of failing patients who complain of exercise intolerance and muscle fatigability (18, 19, 29). This improvement might be due in part to increased eNOS expression and release of NO by endothelium (52). Thus NO could be one of the mechanisms that contribute to physical fitness.

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The aim of this study was to investigate the role of eNOS in physical activity as well as the contribution of eNOS to adaptation of muscle energy metabolism to exercise conditions. We used mice deficient in eNOS (eNOS−/−) that were housed with voluntary wheel running. Our data suggest that eNOS is important for the level of physical activity as well as for the rate of gain of suitable physical capacity.

METHODS

Experimental protocol. Ten-month-old C57BL wild-type (WT) and eNOS−/− (15) mice were divided into active and sedentary groups. Voluntary physical activity of active animals (7 WT and 7 eNOS−/−) placed in cages equipped with running wheels was assessed for 8 wk; sedentary groups (6 WT and 7 eNOS−/−) were housed during this time in cages without wheels. At the end of the experimental period, animals were taken from their cages and at least 30 min later (to avoid possible acute consequences of the exercise for active animals) were anesthetized by an intraperitoneal Pentothal Sodium injection and killed. Cardiac, soleus (slow twitch, oxidative), plantaris (fast twitch, mixed), and the superficial part of gastrocnemius (fast twitch, glycolytic) muscles were isolated, frozen rapidly, and kept at −80°C.

Voluntary activity. Voluntary physical activity of animals was determined by use of the running wheels, which were connected to a direct current (DC) generator allowing slight loading of the wheel (25 × 10−3 N-m) at mean maximal speed and continuous recording of the output voltage of the DC generator on a personal computer. Instantaneous speed was calculated from the voltage and the resistive load on the DC generator and allowed us to calculate the daily distance. The total distance divided by the number of times that each mouse used the wheel gave the distance per run. All daily parameters were averaged over each week for each animal. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Enzyme analysis. Tissue samples were weighed, homogenized in an ice-cold buffer (50 mg wet wt/ml) containing (in mM) 5 HEPES (pH 8.7), 1 EGTA, and 1 dithiothreitol with Triton X-100 (0.1%), and an ice-cold buffer (50 mg wet wt/ml) containing (in mM) 5 HEPES, 10 potassium chloride, 1 magnesium chloride, 1 dithiothreitol with Triton X-100 (0.1%), and 1 EGTA. The mixture containing 0.1% Tween 20 and 1% albumin at room temperature, the protein extracts (20 and 40 μg) were separated by SDS-PAGE with 8% polyacrylamide and blotted onto nitrocellulose membrane. After 2 h of blocking in low-fat milk, the membranes were incubated overnight at 4°C in the presence of iNOS antibodies. Horseradish peroxidase conjugated to goat anti-rabbit IgG was used as secondary antibody. Immunoreactivity was detected by ECL.

Statistical analysis. All data are expressed as means ± SE. One-way or two-way ANOVA for repeated measurements was used to assess the effects of eNOS and voluntary activity, followed by a Newman-Keuls post hoc test when appropriate. Values of P ≤ 0.05 were considered significant.

RESULTS

Voluntary exercise. Figure 1 represents the weekly averaged daily running distance (Fig. 1A), maximal speed (Fig. 1B), and distance per run (Fig. 1C) over 8 wk as a function of time. As can be seen, all parameters were considerably lower in eNOS−/− mice. The running distances averaged for all exercise periods were almost two times lower for eNOS−/− (4.09 ± 0.42 km/day) than for WT (7.4 ± 0.42 km/day; P < 0.01). The averaged maximal speed of running was also lower in eNOS−/− mice as was as low as one-half (10.4 ± 2.2 m) that for the WT group (20.5 ± 2.3 m; P < 0.01). As can be seen from Fig. 1C, distance per run increased progressively during the exercise period in both groups. This parameter, however, rose much faster in the WT group than in the eNOS−/− group, as evidenced by the significant (P < 0.001) interaction between two parameters, genotype and time, shown by two-way ANOVA for repeated measurements. This means that eNOS−/− mice cannot augment their endurance characteristics as early as WT animals do. However, in both groups a significant improvement of all parameters from the first week to the last week of activity was observed.

Effects of eNOS knockout and voluntary exercise on anatomic parameters. Data given in Table 1 show that both voluntary training and absence of eNOS affect body, heart, and soleus muscle weights. The sedentary eNOS−/− mice pre-

![Fig. 1. Voluntary activity of control (○) and endothelial NO synthase (eNOS)−/− (●) mice expressed as daily distance (A), maximal speed (B), and distance per run (C) over the 8-wk period. *P ≤ 0.05, **P ≤ 0.01 vs. control; †first week when the parameter starts to be significantly different from its initial value.](http://ajpheart.physiology.org/)

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sented 48% higher body weight compared with the sedentary WT mice. Voluntary training induced a 19% weight loss in mutant animals, whereas no change was observed for WT mice. Two-way ANOVA analysis showed a significant interaction between eNOS deficiency and training (P < 0.05) for body weight. This indicates that physical activity is able to diminish the eNOS knockout-induced excessive weight. Relative (expressed vs. tibia length) and absolute heart weight increased in knockout animals. Physical activity induced cardiac hypertrophy in WT mice and further increased hypertrophy in mutant animals compared with their sedentary mates. As to skeletal muscle, soleus relative weight was 18% (P < 0.08) lower in the sedentary eNOS−/− mice. Voluntary exercise increased absolute and relative soleus weight by 20% in WT mice, whereas no increase was observed in eNOS−/− mice. Plantaris muscle weight was affected neither by absence of eNOS nor by physical activity.

Effects of eNOS knockout and voluntary exercise on muscle energy metabolism. Tables 2–4 and Fig. 2 show the enzymatic activities of CS (marker of tricarboxylic acid cycle in mitochondria), COX (marker of respiratory chain in mitochondria), CK and AK (energy transfer kinases), as well as LDH (marker of glycolytic chain) in different muscles. In the heart (Table 2), in general neither eNOS deficiency nor physical activity induced considerable changes in energy metabolism enzyme activities, except that exercise increased COX activity by 39% in WT animals. Interestingly, exercise did not induce any increase in this enzyme activity in mutant mice. We also marked a slight increase in M-LDH isoform activity in eNOS−/− sedentary mice.

In soleus muscle (Fig. 2), however, eNOS deficiency induced a decrease in the activity of the mitochondrial respiration chain index, COX, and an increase in LDH activity. Other than a 27% (P < 0.08) increase in CS activity in WT mice, physical training did not produce significant changes in enzyme activities. However, in eNOS−/− mice, exercise led to multiple alterations in energy metabolism activities. We found that COX, CK, and CS activities were reduced by 36% (P < 0.05), 24% (P < 0.01), and 37% (P < 0.06), respectively, compared with their sedentary mates. Western blot analysis of COX subunit VI protein (Fig. 3) showed that this reduction of enzyme activity was associated with a decrease in protein content, although this decrease was not statistically significant. Exercise in mutant mice significantly decreased MM-COX activity by 32%, whereas it increased mitochondrial COX activity by 149% compared with sedentary eNOS−/− mice (Table 3).

In gastrocnemius muscle (Table 4), eNOS deficiency induced a significant increase in COX (139%) and LDH (51%) activities. As in soleus muscle, physical training did not alter metabolite enzyme profile in WT mice but reduced COX activity by 44% in eNOS−/− mice. Two-way ANOVA showed a borderline interaction (P < 0.08) between eNOS−/− and voluntary physical activity COX activity. This evidences a specific effect of exercise on COX activity in the gastrocnemius of eNOS−/− mice.

Expression of INOS in soleus muscle. As eNOS deficiency altered mainly soleus muscle, we checked whether a compensatory iNOS expression occurred that could be involved in the alterations found. Western blot analysis of soleus muscle showed no expression of the iNOS isoform in sedentary or active eNOS−/− mice. As to nNOS, it was shown previously that soleus muscle has a relatively low amount of this isoform and there are no differences in expression of nNOS between

Table 1. Anatomic data

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>eNOS−/−</th>
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</thead>
<tbody>
<tr>
<td>Initial BW, g</td>
<td>35±1</td>
<td>48±1</td>
</tr>
<tr>
<td>Final BW, g</td>
<td>33±1</td>
<td>33±1</td>
</tr>
<tr>
<td>TL, cm</td>
<td>1.84±0.01</td>
<td>1.86±0.01</td>
</tr>
<tr>
<td>HW, g</td>
<td>171±4</td>
<td>215±6°</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>5.21±0.13</td>
<td>6.62±0.17</td>
</tr>
<tr>
<td>HW/TL, mg/cm</td>
<td>93±2</td>
<td>116±3°</td>
</tr>
<tr>
<td>SW, mg</td>
<td>12.9±0.6</td>
<td>15.7±1.1°</td>
</tr>
<tr>
<td>SW/BW, mg/g</td>
<td>0.39±0.02</td>
<td>0.48±0.04°</td>
</tr>
<tr>
<td>PW/BW, mg/g</td>
<td>7.05±0.35</td>
<td>8.40±0.60°</td>
</tr>
<tr>
<td>PW/TL, mg/cm</td>
<td>13.8±0.4</td>
<td>13±10</td>
</tr>
</tbody>
</table>

Values are means ± SE. WT, wild-type mice; eNOS−/−, endothelial NO synthase-null mice; BW, body weight; HW, heart weight; TL, tibia length; SW, soleus weight; PW, plantaris weight. Statistical significance: *P ≤ 0.01, °P ≤ 0.001 sedentary eNOS−/− vs. sedentary WT; †P ≤ 0.05, ‡P ≤ 0.01, active WT vs. sedentary WT; §P ≤ 0.05, ¶P ≤ 0.01 active eNOS−/− vs. sedentary eNOS−/−.

Table 2. Activities of energy metabolism enzymes in ventricular muscle

<table>
<thead>
<tr>
<th>Energetic enzyme</th>
<th>WT</th>
<th>eNOS−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>183±20</td>
<td>182±22</td>
</tr>
<tr>
<td>Cytochrome-c oxidase</td>
<td>420±46</td>
<td>585±33°</td>
</tr>
<tr>
<td>CK</td>
<td>498±47</td>
<td>441±45</td>
</tr>
<tr>
<td>Mitochondrial CK</td>
<td>112±19</td>
<td>97±17</td>
</tr>
<tr>
<td>MM-CM</td>
<td>375±29</td>
<td>325±36</td>
</tr>
<tr>
<td>MB-CM</td>
<td>10.4±4.7</td>
<td>19.2±7.9</td>
</tr>
<tr>
<td>BB-CM</td>
<td>5.2±2.4</td>
<td>9.6±3.9</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>280±36</td>
<td>291±26</td>
</tr>
<tr>
<td>LDH</td>
<td>204±18</td>
<td>255±17</td>
</tr>
<tr>
<td>LDH-H(-M)</td>
<td>122±2</td>
<td>107±8</td>
</tr>
<tr>
<td>LDH-M</td>
<td>82±3</td>
<td>79±10</td>
</tr>
<tr>
<td>LDH-H/-M</td>
<td>1.57±0.7</td>
<td>1.4±0.12</td>
</tr>
</tbody>
</table>

Values (in IU/g wet wt) are means ± SE. CK, creatine kinase; MM-CM, MB-CM, BB-CM, MM, MB, and BB isoforms of CK; LDH, lactate dehydrogenase; LDH-H, LDH-M, H and M isoforms of LDH. Statistical significance: *P ≤ 0.05, sedentary eNOS−/− vs. sedentary WT; †P ≤ 0.05, active WT vs. sedentary WT.

Table 3. Activities of CK and LDH isoenzymes in soleus muscle

<table>
<thead>
<tr>
<th>Energetic enzyme</th>
<th>WT</th>
<th>eNOS−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial CK</td>
<td>34±4</td>
<td>61±13</td>
</tr>
<tr>
<td>MM-CM</td>
<td>1,048±88</td>
<td>956±44</td>
</tr>
<tr>
<td>LDH-H</td>
<td>61±8</td>
<td>57±9</td>
</tr>
<tr>
<td>LDH-M</td>
<td>61±4</td>
<td>49±3</td>
</tr>
<tr>
<td>LDH-H/-M</td>
<td>0.99±0.08</td>
<td>1.11±0.12</td>
</tr>
</tbody>
</table>

Values (in IU/g wet wt) are means ± SE. Statistical significance: *P ≤ 0.05, sedentary eNOS−/− vs. sedentary WT; †P ≤ 0.05, active eNOS−/− vs. sedentary eNOS−/−.
Table 4. Activities of energy metabolism enzymes in gastrocnemius muscle

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 6)</th>
<th>Active (n = 7)</th>
<th>eNOS−/− (n = 7)</th>
<th>Active (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>16±2</td>
<td>19±3</td>
<td>16±1</td>
<td>20.3±1.5</td>
</tr>
<tr>
<td>Cytochrome-c oxidase</td>
<td>3.51±0.72</td>
<td>3.06±0.68</td>
<td>8.42±0.92b</td>
<td>4.71±1.12b</td>
</tr>
<tr>
<td>CK</td>
<td>2.64±228</td>
<td>2.618±252</td>
<td>3.14±0.242</td>
<td>2.656±206</td>
</tr>
<tr>
<td>Mitochondrial CK</td>
<td>1.95±1.95</td>
<td>12.7±8.2</td>
<td>4.77±2.86</td>
<td>9.4±2.5</td>
</tr>
<tr>
<td>MM-CCK</td>
<td>2.64±228</td>
<td>2.605±246</td>
<td>3.135±249</td>
<td>2.652±204</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>699±104</td>
<td>680±107</td>
<td>778±46</td>
<td>583±43</td>
</tr>
<tr>
<td>LDH</td>
<td>336±18</td>
<td>262±47</td>
<td>509±34</td>
<td>315±39</td>
</tr>
<tr>
<td>LDH-H</td>
<td>10.8±1.7</td>
<td>10.5±3.6</td>
<td>21±5</td>
<td>18.5±2.5</td>
</tr>
<tr>
<td>LDH-M</td>
<td>325±15</td>
<td>251±45</td>
<td>488±33</td>
<td>296±37</td>
</tr>
<tr>
<td>LDH-H/M</td>
<td>0.025±0.004</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>0.05±0.01</td>
</tr>
</tbody>
</table>

Values (in IU/g wet wt) are means ± SE. Statistical significance: *P ≤ 0.05, bP ≤ 0.01, sedentary eNOS−/− vs. sedentary WT; †P ≤ 0.05, active eNOS−/− vs. sedentary eNOS−/−.

DISCUSSION

We recently showed (40) that knocking out eNOS induces an important reduction of mitochondrial oxidative capacity specifically in slow-twitch soleus muscle. In the present study, the physical activity of eNOS−/− mice and, further, the effects of voluntary exercise on the key metabolic enzymes in cardiac and skeletal muscles of these animals were evaluated.

We found that all parameters representing physical capacity were lower in eNOS−/− mice compared with their WT mates. Daily distance and distance per run represented only about one-half the respective parameter levels in WT animals. Interestingly, distance per run, a parameter reflecting mostly fatigue resistance, rose progressively during the exercise period, but the rate of this rise was significantly lower in mutant animals. This result suggests that eNOS is important for exercise adaptation.

The data obtained are in line with recent studies showing the importance of NO in physical performance. Rats fed by a NOS inhibitor, nitro-L-arginine methyl ester (L-NAME), were shown to decrease their walking speed progressively (53). Similarly, Maxwell et al. (37) demonstrated that L-NAME in mice reduced various indexes of exercise capacity (maximal oxygen consumption, anaerobic threshold, aerobic work). However, L-NAME inhibits NO production by all NOS isoforms, whereas our work demonstrates an important role of eNOS.

To elucidate possible mechanisms of specifically eNOS-derived NO influence on voluntary physical activity, we evaluated the anatomic profile and metabolic enzyme activities in muscles of eNOS−/− mice. Sedentary mutant mice were characterized by an excessive body weight. This overweight was not associated with any increase in skeletal muscle weight (soleus weight was even diminished); thus one may suggest that eNOS knockout is followed by an increase in adipose tissue. Recently, some metabolic disorders, which might be implicated in the body fat accumulation, were reported in eNOS−/− mice (10). It should be pointed out that this overweight is age dependent because younger (14 wk old) mutant animals demonstrated even lower body weight than WT mice (48).

The excessive body weight in eNOS−/− mice was significantly decreased by voluntary exercise. At the same time, physical activity did not decrease skeletal muscle weight, suggesting that this was adipose tissue mass, which was reduced by exercise. Interestingly, exercise did not diminish body weight of WT animals despite the fact that WT animals had significantly higher physical activity than eNOS−/− mice. Therefore, it is reasonable to suppose that exercise activates specifically those mechanisms that are altered in the absence of eNOS-derived NO. It is well known that exercise induces many responses, including activation of a number of hormonal systems as well as the autonomic neural system. One good candidate is improved glucose uptake at the level of skeletal muscles (20), which is probably able to overcome the increased insulin resistance known to exist in eNOS-deficient muscles (10, 55).
The body weight elevation described here might be among the causes leading to reduced voluntary physical activity in mutant mice. The progressive weight loss during 8 wk of the experiments might explain the slight improvement of physical capacity in eNOS−/− mice, even if the progress was slower than in WT animals. On the other hand, the weight loss probably was not the only reason for the performance improvement because the improvement of exercise parameters in WT animals was not associated with any decrease in body mass.

eNOS−/− mice showed a significant cardiac hypertrophy obviously due to sustained hypertension, well known in these animals (23, 48). Voluntary exercise also induced an expected increase in cardiac mass. The effects of exercise and NOS deficiency on cardiac hypertrophy seem to be additive, as can be seen from a very high heart weight in active eNOS−/− mice.

The cardiac hypertrophy found in mutant mice was not associated with changes in energy metabolism profile in ventricular muscle. Neither mitochondrial enzymes nor enzymes involved in intracellular energy transfer (CK and AK) were modified. The only exception was a slight increase in LDH activity. This result confirms our recent data (40) showing that the absence of eNOS does not change cardiac energy metabolism. Voluntary exercise also did not modify cardiac energetics in either group (the only exception was some increase in COX activity in active WT animals). Thus, in general, energy metabolism in the heart was preserved in both sedentary and active mice under conditions of eNOS knockout, so that the low physical activity of eNOS−/− hearts even demonstrates augmented inotropy (2, 35). All these data are evidence against a possible contribution of impaired cardiac function to the decreased voluntary physical activity in the absence of eNOS. However, the question of whether the eNOS knockout could induce cardiac alterations that are able to limit high exercise capacities needs further investigation.

In contrast to the preserved cardiac enzyme activities, the energetics in oxidative skeletal muscle was markedly impaired in eNOS−/− animals. These data are in accordance with our previous study (40), in which multiple energy metabolism alterations in eNOS knockout mice were found specifically in oxidative skeletal muscle. It is noteworthy that in the present study these alterations in sedentary mice were less important. For example, we did not detect any change in CS activity; similarly, the decrease in CK activity was rather moderate. For example, we did not detect any change in CS activity; similarly, the decrease in CK activity was rather moderate. These differences could probably be explained by the different ages of animals used in our studies. In the previous study we used much older mice (18 mo), and it is reasonable to suggest that the absence of eNOS-derived NO could potentiate age-related processes impairing the metabolism such as decreased angiogenesis (45, 46) or apoptosis. Recent studies show that endothelial NO is an inhibitor of caspase 3, a key mediator of apoptosis (39), whereas inhibition of NOS potentiates apoptosis (54).

A very important result of the present study is a considerable impairment of energy metabolism in soleus muscle of active mutant animals. Mitochondrial marker enzymes and cytosolic CK activities were markedly diminished in these animals compared with sedentary eNOS−/− or active WT mice. Interestingly, the mitochondrial CK increase in active mice was not attenuated by the eNOS deficiency, which suggests that expression of this enzyme is regulated by mechanisms different from those controlling the expression of MM-CK or other mitochondrial enzymes.) These alterations were associated with the absence of exercise-induced increase in muscular mass, well shown in WT animals. Thus, in the absence of eNOS, exercise not only has no beneficial effects on impaired energy metabolism in oxidative skeletal muscle but even worsens the energetics. Yet it is generally accepted that voluntary activity elevates skeletal muscle oxidative capacity, although sometimes the positive effects are limited (5, 33, 47, 57). Therefore, one may suggest that eNOS-derived NO is necessary for maintenance of the energy metabolism profile in oxidative skeletal muscle under exercise conditions. Nevertheless, the alterations in the energy metabolism enzymatic profile in this muscle do not necessarily block the capacity for adaptation to moderate exercise, as can be seen from the progressive physical activity level in mutant mice.

The mechanisms that are activated in the absence of eNOS during exercise and lead to the energetics impairment are not known at present. They could be related to altered vasoreactivity and/or angiogenesis because both processes are known to depend on eNOS function (17, 37, 49). Exercise-induced hyperproduction of reactive oxygen species (for review, see Ref. 25) associated with downregulation of antioxidant systems in eNOS−/− animals (12) could also be involved. Finally, as suggested in our previous report (40), the changes in energetic profile and decrease in muscular mass specifically in oxidative skeletal muscle might be related to accelerated apoptosis. Exercise is a factor activating apoptosis in muscle (for review, see Ref. 44), and in the absence of protective effects of eNOS-derived NO this effect could be more intense.

As already shown by us previously, eNOS knockout has much lesser effects on glycolytic gastrocnemius muscle than on oxidative skeletal muscle. Mutant gastrocnemius muscle demonstrated an elevation in the M isoform of LDH, but physical activity decreased this enzyme as expected in response to exercise (16, 28). An increase in COX activity was also detected in mutant gastrocnemius, but this elevation was modest if one takes into account the very low absolute mitochondrial content in glycolytic muscle. This small absolute increase in COX activity was completely reversed by voluntary activity. The relative resistance of glycolytic muscle to eNOS deficiency may be explained by lower eNOS expression (30, 34) and relatively lower sensitivity of skeletal glycolytic fiber blood perfusion to NO production (38).

The alterations in energy metabolism profile found in oxidative skeletal muscle might be responsible, at least in part, for the low physical activity of the mutant animals. These results allow us to suggest that downregulation of eNOS may contribute to the exercise intolerance and fatigue in different pathologies including heart failure, which is characterized by decreased eNOS expression (9). Importantly, beneficial or deleterious effects of exercise may depend on the eNOS level in skeletal muscle. Among many other effects, physical activity increases eNOS expression in muscle, and this in turn could favor the positive influence of exercise. Conversely, if for some reason eNOS expression is inhibited, the beneficial effects of
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