Training at high exercise intensity promotes qualitative adaptations of mitochondrial function in human skeletal muscle

Frédéric N. Daussin,1,2* Joffrey Zoll,1,2* Elodie Ponsot,1,2 Stéphane P. Dufour,1,2 Stéphane Doutreleau,1,2 Evelyne Lonsdorfer,1,2 Renée Ventura-Clapier,3,4 Bertrand Mettauer,1,2,5 François Piquard,1,2 Bernard Geny,1,2 and Ruddy Richard1,2

1Physiology and Functional Explorations Department, Centre Hospitalier Régional Universitaire of Strasbourg, Strasbourg; 2Physiology Department, Faculty of Medicine, University Louis Pasteur, Equipe d’Accueil 3072, Strasbourg; 3Institut National de la Santé et de la Recherche Médicale, U769, Châtenay-Malabry; 4University Paris-Sud, Châtenay-Malabry; and 5Cardiology Department, Civil Hospital, Colmar, France

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Daussin FN, Zoll J, Ponsot E, Dufour SP, Doutreleau S, Lonsdorfer E, Ventura-Clapier R, Mettauer B, Piquard F, Geny B, Richard R. Training at high exercise intensity promotes qualitative adaptations of mitochondrial function in human skeletal muscle. J Appl Physiol 104: 1436–1441, 2008. First published February 21, 2008; doi:10.1152/japplphysiol.01135.2007.—This study explored mitochondrial capacities to oxidize carbohydrate and fatty acids and functional optimization of mitochondrial respiratory chain complexes in athletes who regularly train at high exercise intensity (ATH, n = 7) compared with sedentary (SED, n = 7). Peak O2 uptake (V̇O2max) was measured, and muscle biopsies of vastus lateralis were collected. Maximal O2 uptake of saponin-skinned myofibers was evaluated with succinate/NADH, palmitoyl carnitine (V̇Pyr), and N,N,N’,N’-tetramethyl-p-phenylenediamine dihydrochloride (V̇TMPD), respectively. V̇O2max was higher in ATH than in SED (57.8 ± 2.2 vs. 31.4 ± 1.3 ml·min⁻¹·kg⁻¹, P < 0.001). V̇GM was higher in ATH than in SED (8.6 ± 0.5 vs. 3.3 ± 0.3 μmol O2·min⁻¹·g dry wt⁻¹, P < 0.001). V̇Pyr was higher in ATH than in SED (8.7 ± 1.0 vs. 8.5 ± 0.2 μmol O2·min⁻¹·g dry wt⁻¹, P = 0.05), whereas V̇TMPD was not significantly different (5.3 ± 0.9 vs. 4.4 ± 0.5 μmol O2·min⁻¹·g dry wt⁻¹). V̇S was higher in ATH than in SED (11.0 ± 0.6 vs. 6.0 ± 0.3 μmol O2·min⁻¹·g dry wt⁻¹, P < 0.001), as well as V̇TMPD (20.1 ± 1.0 vs. 16.2 ± 3.4 μmol O2·min⁻¹·g dry wt⁻¹, P < 0.05). The ratios V̇S/V̇GM (1.3 ± 0.1 vs. 2.0 ± 0.1, P < 0.001) and V̇TMPD/V̇GM (2.4 ± 1.0 vs. 5.2 ± 1.8, P < 0.01) were lower in ATH than in SED. In conclusion, comparison of ATH vs. SED subjects suggests that regular endurance training at high intensity promotes the enhancement of maximal mitochondrial capacities to oxidize carbohydrate rather than fatty acid and induce specific adaptations of the mitochondrial respiratory chain at the level of complex I.

metabolism; exercise training

SKELETAL MUSCLE IS A HIGHLY malleable tissue, capable of pronounced metabolic and morphological adaptations in response to contractile activity (i.e., exercise) (17). Endurance training induces marked metabolic and structural adaptations in skeletal muscle, with an increase in mitochondrial mass and capillary density (19). In parallel with these quantitative im-

* F. N. Daussin and J. Zoll contributed equally to this work.

Address for reprint requests and other correspondence: J. Zoll, Service de Physiologie et d’Explorations Fonctionnelles, Hôpital Civil, 1 Place de l’Hôpital, BP 426, F-67091 Strasbourg Cedex, France (e-mail: joffrey.zoll@medecine.u-strasbg.fr).

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drial respiratory chain complexes (13), which could ameliorate the cellular homeostasis during exercise. However, to date, no study explored the possible adaptations of mitochondrial respiratory chain complexes in human skeletal muscle when muscular activity is highly increased chronically. We hypothesized that, concomitantly with a higher capacity to oxidize CHO, there could be some specific adaptations in the activities of the respiratory complexes in athletes who regularly train at high intensities.

Therefore, this study explored qualitative adaptations at two crucial steps of the mitochondrial machinery in skeletal muscle of athletes (ATH) who trained at high intensity vs. sedentary (SED) human subjects throughout a cross-sectional study. 1) We explored the intrinsic functional capacities of mitochondria to oxidize FA and CHO to determine the long-term adaptations of potentialities for fuel preferences. 2) We investigated the specific adaptations at the level of the mitochondrial respiratory chain complexes.

METHODS

Subjects

Seven SED individuals and seven ATH, all men, participated in the study (Table 1). All subjects were informed about the potential risks associated with the experiment before giving their written consent to participate. ATH were engaged in a regular training schedule comprising five training sessions per week for more than 2 yr, including two weekly training sessions performed at high intensity. Their respective individual training schedule remained unaltered during the 3 wk preceding the experimental period. The investigation was approved by the Consultant Committee on Human Protection from Biomedical Research of Strasbourg, in accordance with the French Law and with the Declaration of Helsinki. It is important to note that the results are obtained with men, and they cannot be generalized to women.

Study Design

Incremental exercise tests. Each subject performed an incremental exercise test (IET) to exhaustion to determine the peak power output, peak oxygen uptake (V˙O2max), and ventilatory thresholds. IET were performed in an upright position on an electronically braked cycle ergometer (Medifit 100S, Belgium). Pedaling frequency was 60–70 revolutions/min and was maintained constant during the test. We used the Hansen equations (15) to determine the maximal theoretical power output of the cycle. We explored the intrinsic functional capacities of mitochondria to oxidize FA and CHO to determine the long-term adaptations of potentialities for fuel preferences. We investigated the specific adaptations at the level of the mitochondrial respiratory chain complexes.

Table 1. Group characteristics

<table>
<thead>
<tr>
<th></th>
<th>SED</th>
<th>ATH</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>41.1±2.4</td>
<td>35.9±4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.9±9.9</td>
<td>72.6±3.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179±3</td>
<td>181±2</td>
</tr>
<tr>
<td>%Predicted V˙O2max</td>
<td>89±4</td>
<td>155±4*</td>
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Values are means ± SE. SED, sedentary subjects; ATH, athletic subjects. %predicted V˙O2max peak oxygen uptake as percentage of the predicted maximum oxygen uptake for SED. *P ≤ 0.001 vs. SED.

Ventilatory parameters. During the tests, V˙O2 was measured on a breath-by-breath basis using an open-circuit metabolic cart with rapid O2 and CO2 analyses (breath-by-breath metabolic measurement, Sensor Medics, MSE, Yorba Linda, CA). V˙O2max was defined as the highest 30-s average V˙O2 value.

Evaluation of ATH training sessions. The average training schedule of ATH is given in Table 2 for the 3 wk preceding the study. All ATH were asked to report their individual training schedule into detailed training logs, including duration, distance, and intensity of each training session, providing both quantitative and qualitative characterization of the overall training load. Duration and intensity of the training sessions were assessed based on the running velocity spread out in four intensity zones: low exercise intensity < LT < moderate exercise intensity < respiration compensatory point < heavy exercise intensity < V˙O2max < severe exercise intensity.

Metabolic parameters. During IET tests, 2-ml blood samples were collected into iced tubes for immediate determination of blood LA concentration (Chiron-Diagnostics Serie 800, Bayer, Puteau, France).

Skeletal muscle biopsy. Vastus lateralis muscle was obtained by the percutaneous Bergstrom technique after local anesthesia was previously described (25). Biopsy was taken 48 h after IET, and subjects were asked to refrain from strenuous exercise during this period. The muscle tissue retrieved was rinsed in ice-cold saline and was used for in situ respiration studies.

In situ study of mitochondrial respiration. The mitochondrial respiration was studied in situ in saponin-skinned fibers. Briefly, fibers were separated under a binocular microscope in solution S at 4°C (see below) and permeabilized in solution S with 50 μg/ml of saponin for 30 min. After being placed in solution R (see below) to wash out adenine nucleotides and create phosphate, skinned separated fibers were transferred in a 3-ml water-jacketed oxygraphic cell (Strathkelvin Instruments, Glasgow, UK) equipped with a Clark electrode, as previously described (21). Solutions R and S contained the following: 2.77 mM CaK2EGTA, 7.23 mM K2EGTA (100 mM free Ca2+), 6.56 mM MgCl2 (1 mM free Mg2+), 20 mM taurine, 0.5 mM DTT, 50 mM potassium-methane sulfonate (160 mM ionic strength), and 20 mM imidazole (pH 7.1). Solution S also contained 5.7 mM Na2ATP, 15 mM creatine-phosphate, while solution R contained 5 mM glutamate, 2 mM malate, 3 mM phosphate, and 2 mg/ml FA free bovine serum. After the experiments, fibers were harvested and dried, and respiration rates were expressed as micromoles of O2 per minute per gram dry weight. Solution R− was similar to solution R without substrates and was used to determined maximal V˙O2 rate for the substrates.

Measurement of the maximal muscular oxidative capacities. The ADP-stimulated respiration above basal V˙O2 (V˙O2b) was measured by addition of 2 mM of ADP. After the determination of the V˙O2b, the maximal fiber respiration rates (V˙max) were measured at 22°C under continuous stirring in the presence of saturating amount of ADP (2 mM) as phosphate acceptor and glutamate-malate as mitochondrial substrates (V˙GM). The acceptor control ratio was V˙GM/V˙O2b and represents the degree of coupling between oxidation and phosphorylation.

Measurements of the substrate oxidative capacities. In separates samples, additions of glycerol-3-phosphate (1.2 mM), pyruvate (1 mM), or palmitoyl-carnitine (135 μM) were done in solution R− in the presence of 2 mM ADP, and maximal V˙O2 rate for these substrates was determined (V˙ODP, V˙PY, and V˙PC, respectively). Malate (2 mM) was added with pyruvate and palmitoyl-carnitine to initiate the Krebs cycle.

Measurement of the respiratory chain complexes. When V˙GM was recorded, electron flow goes through complexes I, III, and IV. Then 4 min after this V˙GM measurement, the complex I was blocked with amytal (2 mM), and then complex II was stimulated with succinate (25 mM). In these conditions, mitochondrial respiration was evaluated by complexes II, III, and IV (V˙3). After that, N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride (TMPD, 0.5 mM) and ascorbate (0.5 mM) were added as an artificial electron donor to cytochrome-c.
Table 2. Training load characteristics of trained subjects

<table>
<thead>
<tr>
<th>Exercise Intensities</th>
<th>Running Time (h:min)</th>
<th>Total Training, %total training time</th>
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<tbody>
<tr>
<td>Low</td>
<td>25.39±2.23</td>
<td>73±2</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.57±0.44</td>
<td>6±2</td>
</tr>
<tr>
<td>High</td>
<td>6.57±0.11</td>
<td>20±1</td>
</tr>
<tr>
<td>Very high</td>
<td>0.14±0.6</td>
<td>1±1</td>
</tr>
<tr>
<td>Total</td>
<td>34.47±2.27</td>
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</table>

Values are means ± SE. Values represent total training load of 3 wk before the study. Intensities zones are as follow: low intensity < lactate threshold < moderate intensity < respiration compensatory point < high intensity < V\textsubscript{O2max} < very high intensity.

In these conditions, cytochrome-c oxidase (complex IV) was studied as an isolated step of respiratory chain (V\textsubscript{TMPD}). The ratios V\textsubscript{S}/V\textsubscript{GM} and V\textsubscript{TMPD}/V\textsubscript{GM} allow exploration of complexes I, II, and IV in ATH vs. SED men.

Statistics. Data are presented as means ± SE. Statistical analyses were performed using Sigma Stat for Windows (version 3.0, SPSS, Chicago, IL). After testing for data distribution normality and variance homogeneity, we used analysis of covariance to adjust group differences for baseline covariates (weight and age). The significance level was set at P < 0.05.

RESULTS

Subject Characteristics

Subjects’ characteristics are given in Table 1. As calculated by Hansen’s formulas, the predicted V\textsubscript{O2max} was not significantly different between SED and ATH. But by design, the V\textsubscript{O2max} was 89 ± 4% of the predicted V\textsubscript{O2max}, as calculated by Hansen’s formula in SED, and 155 ± 4% of predicted V\textsubscript{O2max} in ATH (significantly different from SED, P < 0.001) (41). It is important to notice that, even if ATH trained 79% of total training time at low and moderate intensities, they trained 21% of total training time at very high and severe intensities (Table 2).

Exercise Tests

Table 3 reports the parameters of aerobic performance capacity, as measured during the incremental symptom-limited exercise test. At LT, the V\textsubscript{O2} expressed in relative values was significantly higher in ATH than in SED (P < 0.001). At peak exercise, V\textsubscript{O2max} was much greater in ATH than in SED, according to the fitness differences, but the respiratory exchange ratio remained similar and >1.1, and LA was >8 mmol/l for both groups, suggesting that all of the subjects reached exhaustion during their IET. As expected, the peak LA was higher in ATH (P < 0.05).

Table 3. Gas exchanges during the incremental exercise test

<table>
<thead>
<tr>
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<th>SED</th>
<th>ATH</th>
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<tbody>
<tr>
<td>V\textsubscript{O2} at LT</td>
<td>17.2±1.0</td>
<td>33.2±0.8†</td>
</tr>
<tr>
<td>Peak V\textsubscript{O2}</td>
<td>31.4±1.3</td>
<td>57.8±2.2†</td>
</tr>
<tr>
<td>%HR</td>
<td>92±6</td>
<td>92±5</td>
</tr>
<tr>
<td>RER</td>
<td>1.16±0.04</td>
<td>1.10±0.06</td>
</tr>
<tr>
<td>LA</td>
<td>9.92±2.9</td>
<td>12.4±2.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. V\textsubscript{O2} at LT, oxygen uptake at the ventilatory threshold (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}); peak V\textsubscript{O2}, oxygen uptake per unit body weight (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}); %HR, percentage of theoretical maximal heart rate; RER, respiratory exchange ratio (CO\textsubscript{2} elimination/V\textsubscript{O2}); LA, maximal blood lactate. Significantly different from SED: *P < 0.05; †P < 0.001.

Mitochondrial Substrate Utilization

To compare skeletal muscle oxidative capacities of SED and ATH subjects, we measured the maximal respiratory rates of in situ mitochondria in vastus lateralis samples to determine V\textsubscript{O2max} with different substrates, as well as the functional activities of the respiratory chain complexes. These parameters allow an exhaustive characterization of muscle maximal oxidative capacities with a given substrate, reflecting both mitochondrial density, as well as the functional properties of skeletal muscle mitochondria.

V\textsubscript{O2max} with the different mitochondrial substrates are presented in Fig. 1. V\textsubscript{GDP} was low compared with V\textsubscript{Pyr} and V\textsubscript{PC} and not significantly different among groups. V\textsubscript{GM} was dramatically higher (+156%) in ATH than in SED subjects (8.6 ± 0.5 vs. 3.3 ± 0.3 μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}, P < 0.001). On the other hand, V\textsubscript{PC} was not significantly different between ATH and SED (5.3 ± 0.9 vs. 4.4 ± 0.5 μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}, P = 0.37). V\textsubscript{Pyr} was also significantly higher (+58%) in ATH than in SED subjects (8.7 ± 1.0 vs. 5.5 ± 0.2 μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}, P < 0.05). When comparing the different substrate pathways, V\textsubscript{Pyr} was significantly higher than V\textsubscript{PC} in ATH (+64%, P < 0.05), which was not the case for SED.

We measured maximal activity of the different complexes of the mitochondrial respiratory chain (Fig. 2). With succinate as electron donor for complex II, V\textsubscript{S} was significantly higher in ATH (+84%) than in SED subjects (11.0 ± 0.6 vs. 6.0 ± 0.3 μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}, P < 0.001). Complex IV (TMPD-ascorbate as electron donor) respiratory rates were significantly higher in ATH (+24%) than in SED (20.1 ± 1.0 vs. 16.2 ± 3.4 μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}, P < 0.05). The ratios V\textsubscript{S}/V\textsubscript{GM} and V\textsubscript{TMPD}/V\textsubscript{GM} showed the specific adaptations of the mitochondrial complexes I, II, and IV in skeletal muscle of both groups. V\textsubscript{S}/V\textsubscript{GM} (1.3 ± 0.1 vs. 2.0 ± 0.1, P < 0.001), as well as V\textsubscript{TMPD}/V\textsubscript{GM} (2.4 ± 1.0 vs. 5.2 ± 1.8, P < 0.01) were significantly lower in ATH than in SED, suggesting that the excess of complexes II and IV activities compared with complex I activity was proportionally lower in ATH than in SED.

Fig. 1. Maximal mitochondrial respiration (V\textsubscript{O2max}) of saponin-skinned fibers of skeletal muscle of sedentary subjects (SED; open bars) and athletes (ATH; solid bars) obtained with 4 different substrates: V\textsubscript{GDP}, V\textsubscript{PC}, V\textsubscript{GM}, and V\textsubscript{Pyr}. The substrates used were as follows: G3P, glycerol-3-phosphate; PC, palmitoyl carnitine; M, malate; G, glutamate; Pyr, pyruvate. Values are means ± SE. Significantly different from SED: *P < 0.05; ***P < 0.001. §Significantly different from PC+M: P < 0.05.
Regular endurance training induces numerous physiological adaptations, ultimately improving exercise capacity. Previous studies observed alterations in mitochondrial function with endurance training (27, 33, 36, 37, 44, 45). One of the most characteristic adaptations to training is a change in skeletal muscle substrate metabolism (16). Training-induced shift in substrate utilization is classically attributed to the improved muscular oxidative capacities that result from an increase in mitochondrial density (16). However, other factors must also contribute to the training response, as shown in studies in which metabolism (14) or performance was altered (11, 42), despite no change in muscle oxidative capacities. There is a long-standing notion that endurance training causes a shift from predominantly CHO oxidation toward fat oxidation (16). Accordingly, Cogan et al. (9, 10) found that the rate of utilization of plasma glucose is lower in trained than in untrained subjects during moderate- to high-intensity exercise for a given absolute load, and also when the exercise is performed at the same relative (and, therefore, a higher absolute) intensity. For instance, cyclists and triathletes who trained at least 4 times/wk for 2 h or more and had a training history of at least 5 yr show a higher rate of fat oxidation and an increased relative contribution of fat compared with untrained subjects for an exercise at the same relative intensity (60% of VO2max) (21). Conversely, O’Brien et al. (26) demonstrate that, during marathon running at 73.3 or 64.5% of VO2max, CHO is the primary fuel used. In the same way, Brooks and Mercier (6) have hypothesized that there is a “crossover” effect, such that, during acute exercise at high intensity, the rate of glucose utilization is greater in trained than in untrained subjects, and the rate of glucose uptake increases exponentially with increasing exercise intensity.

In this study, we measured maximal mitochondrial respiration using different substrates, which allow the characterization of mitochondrial fuel preferences for aerobic energy production. Interestingly, we found that mitochondrial intrinsic capacities to oxidize pyruvate (i.e., CHO pathway) are significantly higher than the capacity to oxidize palmitoyl carnitine (i.e., lipid pathway) in ATH who regularly trained at high exercise intensity. These results are in accordance with a recent study of Sahlin et al. (33), which observed that both trained and untrained subject present higher capacity to oxidize pyruvate than palmitoyl carnitine. Moreover, the capacity to use palmitoyl car-
nitrine was not different between ATH and SED. This suggests that mitochondria of ATH exhibited a higher capacity to oxidize CHO than lipid, which was not the case in SED. Indeed, in ATH, maximal mitochondrial respiration with palmitoyl carnitine represents only 60% of the maximal oxidative capacities established with pyruvate and glutamate-malate as substrates. These results substantiate the crossover concept, suggesting that the increased use of CHO during heavy exercise training leads to intrinsic muscular mitochondrial adaptations, promoting the utilization of substrates from the CHO pathways. In fact, high- and very-high-intensity training (20 and 1% of their training duration, respectively) may presumably induce specific metabolic adaptations, different from the adaptations following moderate exercise training.

In the same way, the maximal capacity of muscle to transport and phosphorylate glucose during exercise is higher in the trained state, and regular training increases muscle glucose transporter number and hexokinase activity and reduces the intramuscular glucose-6-phosphate concentration during exercise (12). Accordingly, Kjaer et al. (22) reported that the rate of glucose appearance in blood was higher in endurance athletes than in untrained men during exercise at 60–110% of $V_{O2\text{max}}$. We found that the functional capacity of mitochondria to oxidize FA was not different between SED and ATH. These results are in line with a molecular study showing similar amounts of mRNAs coding for enzymes involved in the β-oxidation of long-chain FA in tibialis anterior muscle of trained and untrained subjects (34). It is also in accordance with the fact that our ATH, in their competitions, perform at intensities that elicit >80% of $V_{O2\text{max}}$, which require mainly CHO oxidation as energy source (6, 38). Thus long-term training at high intensities seems to influence the athlete skeletal muscle profile toward increased mitochondrial capacities to oxidize CHO but not lipids.

It is well known that ATH subjects had a significantly higher myosin heavy chain I percentage and a significantly lower myosin heavy chain IIx percentage than SED subjects (45), and it has been shown that slow-twitch fibers (vs. fast-twitch fibers) have higher activities of enzymes involved in FA oxidation (1). Then we could hypothesize that contractile phenotype could also influence the adaptations of muscular oxidative capacities in our ATH. In case of low-intensity training, substrate oxidation seems to be influenced by fiber-type composition, but not by training status (33). Because our ATH who trained at high intensity increased the capacity to oxidize CHO but not FA, we can suggest that training at high intensity, in contrast to low intensity, promotes muscular CHO oxidation.

Our result could appear in contradiction with the generally well-accepted concept that endurance exercise training leads to a shift of skeletal muscle mitochondria toward an increased use of lipids as a substrate source (for review, see Ref. 18). However, we agree that endurance-trained subjects could use more lipids, both at the same absolute and at the same relative exercise intensity (39), and that higher ADP sensitivity with palmitoyl carnitine than that with pyruvate may influence fuel utilization at low rate of respiration in favor of lipid oxidation (33). Our study provides complementary results, suggesting that mitochondrial adaptations are in favor of higher CHO oxidation capacities in the specific case where subjects regularly train at high intensities. Moreover, further investigations in athletes training at different intensities are needed to explore the role of training intensity in the determination of muscle mitochondrial metabolic profile.

Next to the enhanced capacity to oxidize CHO (i.e., pyruvate), we postulated that high-intensity training could induce some alterations at the level of the mitochondrial respiratory chain complexes to increase the control of mitochondrial respiration (32). The calculation of the ratio of mitochondrial complex activities shows the relative contribution of mitochondrial complexes I, II, and IV in skeletal muscle of both groups. The complex II-to-I ratio, as well as complex IV-to-I ratio were significantly lower in ATH than in SED, suggesting an increase complex I activity compared with complex II and IV in ATH subjects. Because complex I is one of the main limiting steps for the mitochondrial metabolic fluxes in skeletal muscle (32), its augmentation could reflect an improvement in the control coefficient of the respiratory chain (31), allowing amelioration in the rate of NADH oxidation, to support higher CHO oxidation rates. All together, we can postulate that these specific mitochondrial adaptations participate in the increased exercise performance during ATH competition.

**Limitations of the Study**

Because ATH carried out both moderate and high exercise intensities, we cannot completely rule out that moderate exercise sessions did not participate in the qualitative mitochondrial muscular adaptations. Nevertheless, because of the fact that it has been shown that moderate exercise training preferentially increases fat oxidation capacity (3, 7, 9, 23, 35), together with the fact that muscle glycogen oxidation increased in relation to exercise intensity (30), we can postulate that the muscular adaptations favoring CHO metabolism observed in our ATH were predominantly induced by the high-intensity training sessions. This work is a cross-sectional study rather than a longitudinal study, and thus we cannot definitively state that the high-intensity part of the training program was responsible for the observed alterations. Indeed, there may be a difference in baseline mitochondrial function (before high-intensity training) that would not be captured by this study and could participate in the difference in the mitochondrial function between SED and ATH. Then a longitudinal study exploring the mitochondrial adaptations following high-intensity training needs to be carried out to completely rule out this possibility.

**Conclusion**

This cross-sectional study suggests that, in addition to higher skeletal muscle oxidative capacities, important qualitative adaptations take place at the level of substrate utilization. In the particular situation where subjects regularly train at high exercise intensity, there seems to be some mitochondrial adaptations favoring the CHO pathway over the lipid pathway for the highest energetic fluxes, and improving the control of mitochondrial metabolic fluxes through improvement of mitochondrial complex I activity compared with complex II and IV.

**GRANTS**

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