Eccentric exercise causes specific adjustment in pyruvate oxidation by mitochondria.

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Abstract

Introduction: The impact of eccentric exercise on mitochondrial function has only been poorly investigated and remains unclear. This study aimed to identify the changes in skeletal muscle mitochondrial respiration, specifically triggered by a single bout of eccentric treadmill exercise. **Methods:** Male adult mice were randomly divided into eccentric (ECC, downhill running), concentric (CON, uphill running) and unexercised control groups (n=5/group). Running groups performed 18 bouts of 5 min at 20 $cm.s^{-1}$ on an inclined treadmill (+ or – 15 to 20°). Mice were sacrificed 48 hours post exercise for blood and quadriceps muscles collection. Deep proximal (red) and superficial distal (white) muscle portions were used for high-resolution respirometric measurements. **Results:** Plasma creatine kinase activity was significantly higher in ECC compared to CON group, reflecting exercise-induced muscle damage ($p<0.01$). The ECC exercise induced a significant decrease in oxidative phosphorylation capacity in both quadriceps femoris parts ($p=0.032$ in proximal portion, $p=0.010$ in distal portion) in comparison with the CON group. This observation was only made for the NADH pathway using pyruvate+malate as substrates. When expressed as a flux control ratio, indicating a change related to mitochondrial quality rather than quantity, this change appeared more prominent in distal compared to proximal portion of quadriceps muscle. No significant difference between groups was found for the NADH pathway with glutamate or glutamate+malate as substrates, for the succinate pathway or for fatty acid oxidation. **Conclusions:** Our data suggest that ECC exercise specifically affects pyruvate mitochondrial transport and/or oxidation 48h post exercise and this alteration mainly concerns the distal white muscle portion. This study provides new perspectives to improve our understanding of the mitochondrial adaptation associated with ECC exercise.

Keywords: Eccentric exercise, Exercise-induced muscle damage, mitochondrial function, oxidative phosphorylation

Introduction

Eccentric (ECC) exercise presents growing interests in sport as well as in several clinical fields (1-3). In contrast to conventional resistance exercise training, which involves both shortening (concentric, CON) and lengthening (ECC) contractions, ECC muscle contractions are very distinct and occur when a force applied to the muscle exceeds the momentary force produced by the muscle itself, resulting in the forced lengthening of the muscle-tendon system while contracting (4). This results in the production of a greater peak tension than in other modes of contraction (5). During the ECC contraction process, the muscle absorbs energy developed by an external load, explaining why eccentric action is also called “negative work” (lengthening) as opposed to concentric “positive work” (shortening) contraction. These types of contraction can be separated in specific training to induce distinct muscle adaptations(6).

Because the metabolic cost and cardio-respiratory stress of ECC contractions has been reported to approximatively one quarter compared to concentric activity at similar intensity, these contractions can achieve highest force with a greatly reduced oxygen requirement (7, 8). For this reason, ECC contractions are an increasingly recognized method for physical rehabilitation in diverse pathologies of the locomotor system, to prevent muscle injuries, as well as to improve muscular strength, power and coordination (reviewed by (9)). It is also particularly attractive for patients with chronic heart and lung diseases (10). Furthermore, it was suggested as a potential intervention to facilitate muscle maintenance with aging (11). This was based on positive effect on muscle mass and strength (reviewed by (12)), whereas contradictory results also suggest no effect (13-15).

Compared to other modes of exercise (i.e., CON or isometric contractions), unaccustomed and/or intense ECC contractions are more frequently pointed as responsible for the apparition of muscle fiber damage and delayed-onset muscle soreness (DOMS), a sensation of deferred and variable intensity stiffness of the muscle affected

(16, 17). A single bout of repeated maximal ECC exercise of the knee extensors has been shown to promote a 40%–50% reduction in maximal strength from baseline levels that persists for several days or even weeks (18, 19). The decrease in force has been associated with several muscle disruptions including general swelling, inflammation, intracellular calcium imbalance and increased production of reactive oxygen species (ROS) (reviewed by (20)). The muscle damage caused by ECC exercise was suggested to be due to the combination of greater force and reduced recruitment of fibers number during ECC contractions, causing a high mechanical stress on the involved structures (21). Even if specific training has shown success in preventing DOMS, there is currently no unified theory available to explain the DOMS phenomenon occurring after ECC exercise.

Whereas regular CON exercise training has well documented positive effect on mitochondrial function in animals (22-24) and in humans (25, 26), studies suggest that ECC exercise has a different effect. In fact, the scarce literature regarding the changes in mitochondrial metabolism occurring in skeletal muscle after unaccustomed ECC exercise point toward unexplained contradictive results. In a recent review of the literature, it was concluded that endurance chronic ECC exercise and acute endurance ECC exercise are not affecting mitochondrial respiration (20). However, the literature presented in this review showed contradictory results on the effect of unaccustomed ECC exercise on mitochondrial function, revealing some defects in mitochondrial respiration within 48 hours after unaccustomed ECC exercise (27-29). In many studies, including the three presenting an effect of ECC exercise on mitochondrial function, there was no comparison with another type of exercise, *e.g.*, CON exercise. Furthermore, depending of the substrate used, the defect could be present (with pyruvate and malate (27-29)) or not (with succinate (29)). Interestingly to us, no studies addressed the impact of acute ECC exercise on specific steps and pathways of mitochondrial oxidative phosphorylation (OXPHOS). In humans, it has also been shown that a single bout of unaccustomed resistance exercise reduces the expression of mitochondrial transcripts, proteins, and mitochondrial DNA copy number in young and old adults (30). Furthermore, in rodents, ECC exercise impairs calcium handling, which is directly linked to mitochondrial function (31), and downregulates the expression of factors involved in mitochondrial biogenesis (27, 32).

Here we suggest to revisit the question and to address three related sub-questions: i) the impact of the substrates used to measure mitochondrial function to investigate multiple electron transfer pathways, ii) the comparison between ECC and CON exercise and iii) the specificity to the proximal or distal portion of the *quadriceps femoris* muscle. The objective is to explain some of the contradictions observed in the literature, and better understand the short-term impact of ECC exercise on mitochondrial function in the muscle. For this purpose, we used a mouse model running on an inclined treadmill (6). The downhill running (DHR) solicits the knee extensor muscles predominantly in an ECC manner, inducing potential muscle damage in this muscle group. In contrast, the uphill running (UHR) recruits the same muscles but in a concentric way, leading to less microlesions. Intermittent bouts (rather than continuous running) were used to maintain a higher speed of running all along the protocol. An improved understanding of the changes in mitochondrial function specifically triggered by ECC muscle contractions, in direct comparison with CON contraction, should provide invaluable information to understand the impact on muscle health and adaptation.

Methods

Animals

Experiments were performed in accordance with the Animal Ethics Committee of the University of Liège (07-703) and were conformed with the policy statement of the American College of Sports Medicine on research with experimental animals. Fifteen male C57BL/J6 mice (10-12 weeks old) were maintained on a 12/12-hour light-dark cycle and received standard food pellets and water *ad libitum*. Mice were provided by the Central Animal Facility of the CHU Sart Tilman (Liège, Belgium). The Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines were carefully followed as well as national and european legislation.

Running protocol

Mice were randomly assigned to an unexercised control group, an uphill running group (CON) or a downhill running group (ECC). Exercised groups underwent an intense running bout on an inclined motor-driven treadmill (Bioseb, Paris, France). Before the exercise session, mice were placed on a stationary individual lane of the treadmill for a few minutes to allow them to become familiar with the running environment. This acclimation period was followed by a 10-min warm-up. The speed was initially fixed at 5 $cm.s^{-1}$ and increased of 1 $cm.s^{-1}$ every 30 s until reaching 20 $cm.s^{-1}$. After a 2 minutes rest period, mice performed 18 running bouts of 5 minutes at 20 $cm.s^{-1}$. Each bout was interspersed with a 2 minutes rest. Mice performed voluntarily the running exercise. If a mouse stopped running, a gentle tap on the mouse back by an investigator was sufficient to stimulate the mouse to keep running. Mice ran the first eight bouts at a grade of + or – 15° and the last ten bouts at a grade of + or – 20°. Positive and negative slopes were used for uphill and downhill exercise, respectively.

Blood and tissue collection

Forty-eight hours after the running exercise, mice were killed by cervical dislocation for blood and muscle collection. The time point was chosen according to previous results showing effect of a single bout of ECC exercise on mitochondrial function in rodents (27, 28). Furthermore, this time point was also reinforced by a study in rats using a combined magnetic resonance imaging and skeletal muscle histopathological analysis to explore the kinetics of ECC exercise-induced injury, which showed maximal disruption 2 days post-exercise with gradual muscle repair continuing until at least day 7 (33). Blood was sampled from the heart using a heparinized syringe and centrifuged at 4,000 rpm for 15 min. Plasma was stored at -80°C for later analysis of creatine kinase activity (See below). The knee extensor muscles (proximal or red portion and distal or white portion; (34-36)) were dissected, cut in small pieces and immediately immersed in ice cold muscle relaxing solution BIOPS (10 mmol/l CaK_2-EGTA, 7.23 mmol/l K_2-EGTA, 20 mmol/l imidazole, 20 mmol/l taurine, 50 mmol/l K-MES, 0.5 mmol/l dithiotreitol, 6.56 mmol/l $MgCl_2$, 5.77 mmol/l ATP and 15 mmol/l

150 phosphocreatine adjusted to pH 7.1; (37)). The fibers were then transferred to BIOPS and mechanically
151 permeabilized with forceps, followed by gentle agitation for 30 min at 4 °C in relaxing solution supplemented
152 with 50 ug.ml^{-1} saponin (37). Fibers were washed for 10 min by agitation in ice-cold mitochondrial respiratory
153 medium MiR05 (0.5 mM EGTA, 3 mM $MgCl_2$-$6H_2O$, 60 mM K-lactobionate, 20 mM taurine, 10 mM
154 KH_2PO_4, 20 mM HEPES, 110 mM sucrose, and 1 g/L BSA essentially fatty-acid free, pH 7.1, (37)). The fibers
155 were then blotted, weighted and immediately used for respirometric measurements (see below).
156
157 *Plasma creatine kinase*
158 Blood samples were drawn from the heart in 5 mL in heparin tubes. Plasma creatine kinase (CK) activity was
159 determined by routine spectrophotometric techniques used in the Department of Clinical Chemistry, using the
160 CK-reagent kit (Roche diagnostic, number 12132672 216) (16, 38).
161
162 *High resolution respirometry*
163 Respirometric measurements were performed at 37 °C using the Oxygraph-2k (OROBOS Instruments,
164 Innsbruck, Austria) using about 2 mg wet weight of permeabilized fibers (mean 2.0 mg, min 0.92 mg, max
165 3.13 mg) to the oxygraph chamber containing 2.1 ml of MiR05. Eight respirometry assays were applied to the
166 permeabilized fibers to evaluate the capacity of electron transfer (ET) pathways and steps and associated
167 dehydrogenases or fatty acid β-oxidation and associated enzymes. Two states were included in each protocol:
168 (1) LEAK respiration in the non-phosphorylated state without ADP, and (2) OXPHOS capacity coupled to
169 phosphorylation of ADP into ATP in the presence of saturating ADP (2.5 mM). The first 5 protocols include
170 the measurement of LEAK and OXPHOS capacity in the presence of substrates feeding electrons into the
171 NADH pathway; pyruvate and malate, glutamate and malate, and glutamate alone, the succinate pathway
172 (Succinate and rotenone) and the complex IV activity (ascorbate and TMPD). For the complex IV, the
173 chemical background with 100 mM sodium azide was subtracted to obtain the activity. The last three protocols
174 allowed the estimation of LEAK and OXPHOS for fatty acid oxidation of long chain (palmitoylcarnitine and
175 malate), medium chain (octanoylcarnitine and malate) and short chain (acetylcarnitine and malate) fatty acids.

In the first three protocols, allowing estimation of NADH pathways, the protocols were continued to add the succinate pathway (NADH and Succinate pathway simultaneously, NS pathway) under OXPHOS state and then under ET state (electron transport system capacity after uncoupling with optimum carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) concentration). This allowed to express the respiratory capacity both in Flux per mass (pmol $O_2.s^{-1}.mg^{-1}$) and as a Flux Control Ratio (*FCR*), normalized for maximal ET capacity in the presence of substrates feeding electrons simultaneously into the NADH and Succinate pathways. Artificial oxygen diffusion limitation was avoided in all protocols by maintaining oxygen levels over 200 μM O_2 (37), and instrumental oxygen background fluxes were calibrated as a function of oxygen concentration and subtracted from the total volume-specific oxygen flux using DatLab software (Version 7.4.0.4, Oroboros Instruments). All chemicals for measurements of mitochondrial function were from the sources suggested in Lassnig et al. (2008) (39).

Integrity of outer mitochondrial membrane

Exogenous cytochrome *c*, added after ADP, exerted a slight effect on OXPHOS capacity. This effect was expressed as a cytochrome *c* control factor (FCF*c*), indicating the control of respiration by externally added cytochrome *c* (40). A value of 0.00 indicates no control of respiration by exogenous cytochrome c, and so complete integrity of the mitochondrial outer membrane. There was no difference between the groups for cytochrome *c* values in distal portion ($p=0.319$, $p=0.864$, $p=0.890$, for the PM, GM, and G protocols, respectively; ANOVA on rank) or proximal portion of the muscle ($p=0.459$, $p=994$, $p=1.000$, for the PMS, GS, and G protocols, respectively; ANOVA on rank). For the distal portion, the mean (min-max) values were the following: 0.02 (0.00-0.08) in the PMS protocol; 0.02 (0.00-0.21) in the GMS protocol; and 0.04 (0.00-0.26) in the GS protocol. For the proximal portion, the mean (min-max) values were the following: 0.01 (0.00-0.09) for the PMS protocol; 0.01 (0.00-0.04) for the GMS protocol; and 0.02 (0.00-0.10) for GS the protocol. Our values validate the integrity of mitochondrial membranes in the fiber preparation, with similar or lower values to previous results in permeabilized skeletal muscle fibers from mice (41) or rats (42).

Data analysis

Graphics were produced using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). Statistical analyses were performed with SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA). The difference between the three groups, within proximal and distal muscle portions (predominantly composed of type I and II fibers, respectively) were analyzed with a one way ANOVA. The criteria for normality and homogeneity of variance were tested for each variable using Shapiro-Wilk and Brown-Forsythe tests, respectively. For the variable that did not pass the conditions, an ANOVA on rank (Kruskal-Wallis) was performed. Data are presented without transformation, as dot plots with the horizontal line representing the mean. A $p<0.05$ was considered significant.

Results

Plasma CK activity is affected significantly by the ECC exercise.

The values of the plasma CK activity are used to indirectly assess the presence of muscle damage (reviewed by (9)). The CK activity was higher in the ECC exercise group compared to the CONC exercise group ($p<0.01$) (Fig. 1).

Eccentric exercise affects early and specifically the pyruvate oxidation within the mitochondria.

The data in Figure 2 showed the OXPHOS capacity relative to the sample mass for the various pathways and substrates associated with the 8 protocols. The OXPHOS capacity in oxygen consumption per mg tissue mass for the NADH pathway in the presence of pyruvate and malate varied significantly between the exercise groups both in proximal and distal portions of the muscle (Fig. 2A): the ECC exercise caused a significant reduction

in the capacity compared to the concentric exercise ($p=0.010$ in distal portion and $p=0.032$ in proximal portion). In contrast, the NADH pathway capacity with glutamate + malate (GM; Fig. 2B) or with glutamate alone (G; Fig. 2C) did not change with the exercise group in either distal ($p=0.394$ with GM and 0.771 with G) or proximal portion of the muscle ($p=0.109$ with GM and $p=0.743$ with G). In both muscle portions, there was also no change associated with the exercise in the Succinate pathway ($p=0.404$ in distal and $p=0.355$ in proximal; Fig. 2D) and the Complex IV ($p=0.560$ in distal and $p=0.063$ in proximal; Fig. 2E). Fatty acid oxidation was also not affected by the exercise for the short-chain fatty acid substrate acetylcarnitine ($p=0.218$ for distal and $p=0.868$ for proximal; Fig. 2F), the long-chain fatty acid oxidation substrate palmitoylcarnitine ($p=0.369$ for proximal; Fig. 2G) or the medium-chain fatty acid substrate octanoylcarnitine ($p=0.281$ in proximal; Fig. 2H).

Because of the significant difference obtained for the NADH pathway in flux per mass, we investigated further with the flux control ratios. *FCR* are normalized to the maximal ET capacity with substrates feeding electrons into the NADH and Succinate (NS) pathways simultaneously. The *FCR* represent the proportional contribution of a specific pathway, and therefore are dictated by the mitochondrial properties rather than the mitochondrial content. The *FCR* for the NADH pathway was changed with exercise but only with pyruvate and malate as substrates, and only in the distal portion ($p<0.001$ for distal and $p=0.659$ for proximal; Fig. 3A). In the presence of glutamate + malate ($p=0.373$ in distal portion and $p=0.233$ in proximal portion; Fig. 3B) or glutamate alone ($p=0.712$ in distal portion and $p=0.753$ in proximal portion; Fig. 3C), no changes were associated with the type of exercise.

Leak and membrane integrity did not vary with exercise.

The *FCR* for LEAK presented in Figure 4 was used as an indicator of coupling of the OXPHOS process, independent from mitochondrial content and OXPHOS capacity (40). A minimum value of 0.0 indicates a fully coupled system, while a value of 1.0 indicate a fully non-coupled system. The *FCR* for LEAK was not

affected by exercise in any of the protocols and muscle portions (in distal portion p=0.193, 0.731 and 0.742 for A, B, and C respectively; in proximal portion p=0.463, 0.969 and 0.989, for A, B, and C, respectively; all ANOVAs except for the GM protocol in distal portion where the data did not pass the condition and were analyzed with an ANOVA on rank).

Discussion

Despite considerable amount of clinical and histological data available, a significant gap still remains to understand the mechanisms mediating the cellular response to muscle damage due to ECC exercise (9). Here we are addressing the role of mitochondrial function, as it was suggested to be an important player, but past studies led to contradictory results. Our experimental design focused on understanding the specific changes in mitochondrial function occurring 48 hours after a single acute bout of ECC exercise. In contrast to previous studies, we examined a large range of OXPHOS pathways and steps in permeabilized fibers from proximal red and distal white portions of the knee extensor muscles. Our results show that 48 hours after one session of ECC exercise, there is a reduction in mitochondrial function. More importantly, the reduction is specific to pyruvate oxidation, and does not affect mitochondrial complexes capacities or fatty acid oxidation. The specific change is also more prominent in the distal portion of the muscle, where it is also significant when the data are expressed relative to the maximal OXPHOS capacity, defining a change not related to mitochondrial quantity but to mitochondrial quality.

The CK activity assay confirmed a strong impact of only one session of ECC exercise on muscle damage at the time point selected in our study. A high level of plasma CK activity is accepted as an indicator of exercise-induced muscle damage (43). Forty-eight hours after exercise, a drastic 4-fold increase in plasma CK activity was measured in the ECC group when compared to the CON exercise groups in our study. An elevation in blood CK activity 24–48 h post-exercise reflecting myofiber membrane disruption is also a consistent finding

after ECC exercise, the magnitude of increase being related to the exercise intensity (reviewed by (20)). Interestingly, the next day plasma CK activity has also been shown to be highly correlated with the muscle fatigue profile during ECC exercise (38). Of important note, we detected high variability in CK activity in our ECC group, as previously detected in humans, some subjects being defined as 'high-responders' without a clear explanation why (44-46).

After confirming the impact of ECC exercises with the CK activity assay, we focused on the study of mitochondrial function. Our results showed for the first time that change in mitochondrial function 48 h after ECC exercise is specific to pyruvate oxidation, while oxidation of several other substrates (glutamate, fatty acids, succinate, or substrates providing electrons directly into complex IV) are unchanged. This points toward changes of pyruvate dehydrogenase complex (PDC) activity or pyruvate transporter rather than mitochondrial complexes or other enzymes associated with it. Previous studies measuring respiration with only one or two groups of substrates did not allow identification of the specific pathway or step modified within the mitochondria.

Our results offer an opportunity to reinterpret and compare previous results from the literature on the effect of ECC exercise on mitochondrial function (reviewed by (20)). If we consider our results and the substrates used in previous studies, we can reconcile most of the contradictory results, and reach different conclusions. Other than our study, the only studies that point toward a decrease in mitochondrial function shortly (0 to 48 hours) after a single bout of acute ECC exercise have also performed the measurement with pyruvate and malate as substrates (27-29). In agreement with our results, all previous results using succinate (29, 47, 48), glutamate and malate (48, 49), or glutamate and malate and succinate (29, 49, 50), did not measure significant change in mitochondrial function. These studies are concurrent with our data showing that the change is specific to pyruvate transport or oxidation into the mitochondria. It is also interesting to note that other studies measuring activity or expression of various enzymes also confirmed that the electron transport system complex I (29), complex II (29), and the glutamate dehydrogenase (16) are not affected by ECC exercise. This also agrees

306 with our conclusions that the change is specific to pyruvate intramitochondrial transport and/or oxidation and
307 does not involve the electron transport complexes or the TCA cycle.
308
309 Only one study in humans (51) and one study in rats (47) presented contradictory results when using pyruvate
310 and malate as substrates, within the 48 hours after unaccustomed ECC exercise. In the study of Walsh,
311 Tonkonogi, Malm, Ekblom and Sahlin (51) in human muscle, cycling was used instead of walking as the
312 exercise and the duration of the training was only a 30 min, compared to 90 min (our study and (27, 28) and
313 120 min (29). The impact of change in type of exercise and shorter duration in the study of Walsh et al. (2001)
314 is also shown by the results of CK which show no significant difference, compared a 4-fold increase associated
315 with ECC exercise in our study. Similarly, the studies of Rattray et al. (28) and Magalhães et al. (29), which
316 both showed significant a decrease in pyruvate oxidation, also showed a significant increase in the CK activity
317 at the same time points. Furthermore, even if a significant increase in CK activity was reached only after heat
318 exposure (27), a strong trend was observed even without the heat treatment (~3-fold increase in CK after ECC
319 exercise), but the variability did not allow to point out a significant difference. Also, in agreement with our
320 interpretation, in the study of Molnar et al. in rats (47), the mitochondrial function was measured only
321 immediately after the acute exercise, at a time point where there was no change in CK activity; no measurement
322 of mitochondrial function was performed 3 and 6 hours after the acute ECC exercise, *i.e.*, the time points
323 showing significant increase in CK activity. Similarly, in Magalhães et al. (29) , the increase in CK and the
324 decrease in pyruvate oxidation was significant only for the time point immediately after the bout of ECC
325 exercise, and none were changed 48 hours after ECC exercise. The same phenomenon was present in the paper
326 by Rattray et al. (27), where the change in CK were more prominent at time 0 (~3-fold) and 48 hours (~2.5-
327 fold) after the ECC exercise, whereas it was more modest 2 hours after (~1.4-fold), and the time point with
328 significant changes in pyruvate oxidation were at 0 and 48 hours. Our results and previous results strongly
329 suggest that pyruvate oxidation in mitochondrial matrix is modified by ECC exercise, but only at time point
330 causing a significant increase in plasma CK activity, a marker for muscle damage.
331

A specific decrease in pyruvate oxidation like what we observed here can be due to a reduction in PDC activity or pyruvate transporter activity. The activity of PDC is regulated by the pyruvate dehydrogenase kinase and the pyruvate dehydrogenase phosphatase. The kinase promotes the phosphorylated and inactive form of PDC, while the active dephosphorylated form of the enzymes is promoted by the phosphatase. Calcium ions (Ca^{2+}) act as important regulator of the PDC activity. In normal circumstances, Ca^{2+} is released from the sarcoplasmic reticulum when the muscle is stimulated, leading to a sarcoplasmic Ca^{2+} overload. In response of this change, the mitochondria rapidly store large amounts of precipitated Ca^{2+}-phosphate (reviewed by (52)). The increase of mitochondrial Ca^{2+} up to low micromolar levels resulting from the uptake leads to activation of the PDC phosphatase, dephosphorylating and powerfully stimulating the PDC activity (53). Even if the increase in intramitochondrial calcium is important during muscle contraction, a mitochondrial calcium overload has been associated with dose-dependent inhibition of pyruvate-supported mitochondrial respiration in the brain and skeletal muscle mitochondria (54-56). Interestingly, unaccustomed ECC exercise has been shown to induce several signs of perturbations in calcium homeostasis including (1) intracellular calcium accumulation (reviewed by Touron et al. (20)), (2) increase Ca^{2+} concentration within the mitochondria (27, 28, 57), (3) decrease in Ca^{2+} uptake from two to 14 days after the session (58). Previous results suggest that the inhibition of pyruvate oxidation linked with high concentration of Ca^{2+} could occur not only through the phosphorylation state of PDC, but other mechanisms could contribute as well. In the study from Pandya et al. (55), the decrease in pyruvate oxidation linked with calcium concentration was in fact not associated with a decrease in PDC or complex I activities. Further studies are needed to examine if the decrease in pyruvate oxidation is linked with increase in mitochondrial calcium, and if so, if a mechanism other than PDC phosphorylation could explain the change in pyruvate oxidation associated with ECC exercise. One possible way would been by affecting pyruvate transport into the mitochondria through the mitochondrial pyruvate carrier, which has already be shown to be regulated in various circumstances (59, 60). Furthermore, calcium overload is associated with opening of the mitochondrial permeability transition pore (mPTP) (61) possibly leading to the activation of cell death signaling. Calcium also increases calpain proteolytic activity which is able to target mitochondrial proteins resulting in mitochondrial function impairment (62). In addition, related or not to calcium overload,

the decrease in PDC activity in ECC exercise could, according to previous studies, lead to excessive RNOS production (63) or to lactate accumulation (53).

Finally, our results are the first to identify the more prominent changes in the the distal portion (principally composed of fast-twitch, type II fibers (64)) compared to the proximal portion (principally composed of slow-twitch, type I fibers (64)) of the quadriceps femoris muscle. Other studies discussed here involved a whole muscle model, without distinction of the muscle portion. Several authors suggested that the skeletal muscle responses to acute or chronic ECC exercise are specific on the muscle fiber type. Indeed, both human and animal studies supported that type II (in particular IIb) muscle fibers are more damaged after high intensity ECC exercise than type I fibers (reviewed by (9)). Interestingly, it was previously suggested that eccentrically biased exercise leads to more fibers displaying an oxidative phenotype (type I and IIa fibers) and less fibers displaying a glycolytic phenotype (type IIb fiber) compared to concentrically biased training. This is the case both in mice (6) and in human (16), and was measured after only 5 sessions of ECC exercises. It is possible that the early impact of ECC exercise, more pronounced in the distal portion of the quadriceps muscle, might contribute to this adaptation on the longer term.

Conclusions

Endurance eccentric training recently appears as a novel training modality (11). Growing evidence has been published over the last decades on its beneficial effects on skeletal muscle and general health in many healthy and clinical populations (9). However, the scarce literature regarding the changes in mitochondrial metabolism occurring in skeletal muscle after ECC exercise justified more investigations. To the best of our knowledge, this is the first study to measure and compare mitochondrial respiration in specific portions of quadriceps femoris after endurance ECC exercise in direct comparison to CON exercise. Interestingly, our data highlighted a reduced pyruvate oxidation 48 hours following a single bout of ECC exercise, while oxidation of several other substrates was unchanged. This modification in mitochondrial function seems to mainly concern the distal white portion of the muscle. Our observations offer the opportunity to reconcile previous

contradictory results and led to new research perspectives. Indeed, further investigations are needed to unravel the molecular mechanisms underlying the reduced pyruvate oxidation following ECC exercise. A specific regulation of pyruvate dehydrogenase complex (PDC) activity or pyruvate transporter activity might be explored.

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Conflict of interests

The authors declare that there is no conflict of interest.

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552 **Figure legends**

553

554 FIGURE 1: Creatine kinase (CK) activity in the plasma of the mice. The three groups correspond to the control
555 (without exercise), and the 48 hours after a session of eccentric and concentric exercises. Data are presented
556 as dot plots with the horizontal lines representing the mean and SEM. P<0.01 are indicated with a symbol **.
557 N = 5 animals per group. An ANOVA on rank was performed.

558

559 FIGURE 2: Oxidative phosphorylation capacity in flux per mass various substrates in the permeabilized fibers
560 from the proximal and distal parts of the knee extensor muscles. The three groups correspond to the control
561 (without exercise), and the 48 hours after a session of eccentric and concentric exercises. Respiratory capacity
562 was for the OXPHOS state, coupled to the production of ATP. The pathways and steps measured include the
563 NADH pathway through complex I in the presence of (A) pyruvate and malate (PM), (B) glutamate and malate
564 (GM), (C) glutamate alone (G), the succinate pathway in the presence of (D) succinate, the complex IV activity
565 (E), and the fatty acid beta-oxidation in the presence of (F) acetylcarnitine and malate (ActCar), (G)
566 palmitoylcarnitine and malate (PalCar), and (H) octanoylcarnitine and malate (OctCar). Data are presented as
567 dot plots with the horizontal lines representing the mean and the SEM. The dots represent the number of fiber
568 preparations. The dots represent the numbers of fiber preparations (n), which is equal to the numbers of animals
569 (N), except in (A) (N=6 for distal portion in control and concentric; N=5 in proximal portion for eccentric,
570 N=4 for procimal portion concentric and distal portion eccentric, N=3 for proximal portion control). Within a
571 muscle portion (proximal or distal), P<0.05 are indicated with a symbol *. All variables were analyzed with
572 one-way ANOVA, except in A (proximal and distal portions) and H (proximal portion), for which the
573 assumption were not met and an ANOVA on rank was performed.

574

575 FIGURE 3: Oxidative phosphorylation capacity expressed as a flux control ratio (*FCR*) in the permeabilized
576 fibers of the proximal and distal parts of the knee extensor muscles. The FCR is normalized for maximal
577 oxidative phosphorylation (OXPHOS) capacity in the presence of substrates feeding electrons simultaneously

into the NADH and succinate pathways. The three groups correspond to the control (without exercise), and the 48 hours after a session of eccentric and concentric exercises. Respiratory capacity was for the OXPHOS state, coupled to the production of ATP. The substrates for measurement of the NADH pathway include (A) pyruvate and malate (PM), (B) glutamate and malate (GM), (C) glutamate alone (G).Data are presented as dot plots with the horizontal lines representing the mean and SEM. Within a muscle portion (proximal or distal), $P<0.01$ are indicated with a symbol **. The numbers of animals are as specified in Fig. 2. All variables passed the assumptions and were analyzed with one-way ANOVA.

FIGURE 4: Mitochondrial coupling in the permeabilized fibers of the proximal and distal parts of the knee extensor muscles. LEAK respiration was determined in the absence of ADP and in the presence of substrates feeding electrons into the NADH pathway, i.e. (A) pyruvate and malate (PM), (B) glutamate and malate (GM), and (C) glutamate alone (G). LEAK respiration is expressed as a flux control ratio (FCR), over the maximal OXPHOS capacity with substrates feeding electrons into both the NADH and the Succinate pathways simultaneously. Data are presented as dot plots with the horizontal lines representing the mean and SEM. There is no significant difference here within a muscle portion (proximal or distal). The numbers of animals are as specified in Fig. 2. All variables were analyzed with one-way ANOVA, except in C (distal portion), for which the assumption was not met and an ANOVA on rank was performed.

597

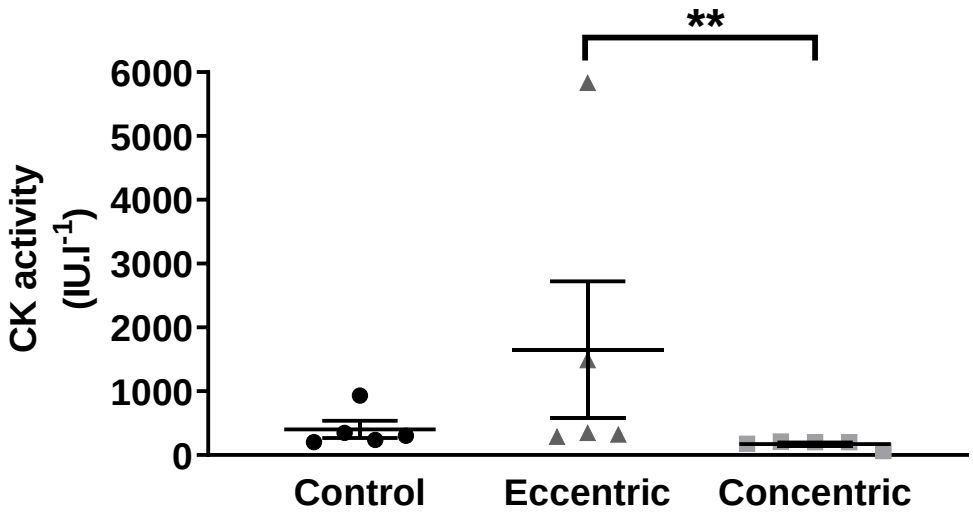
**
6000
5000
4000
3000
2000
1000
0
CK activity
(IU.l-1)
Control
Eccentric
Concentric

FIGURE 1.

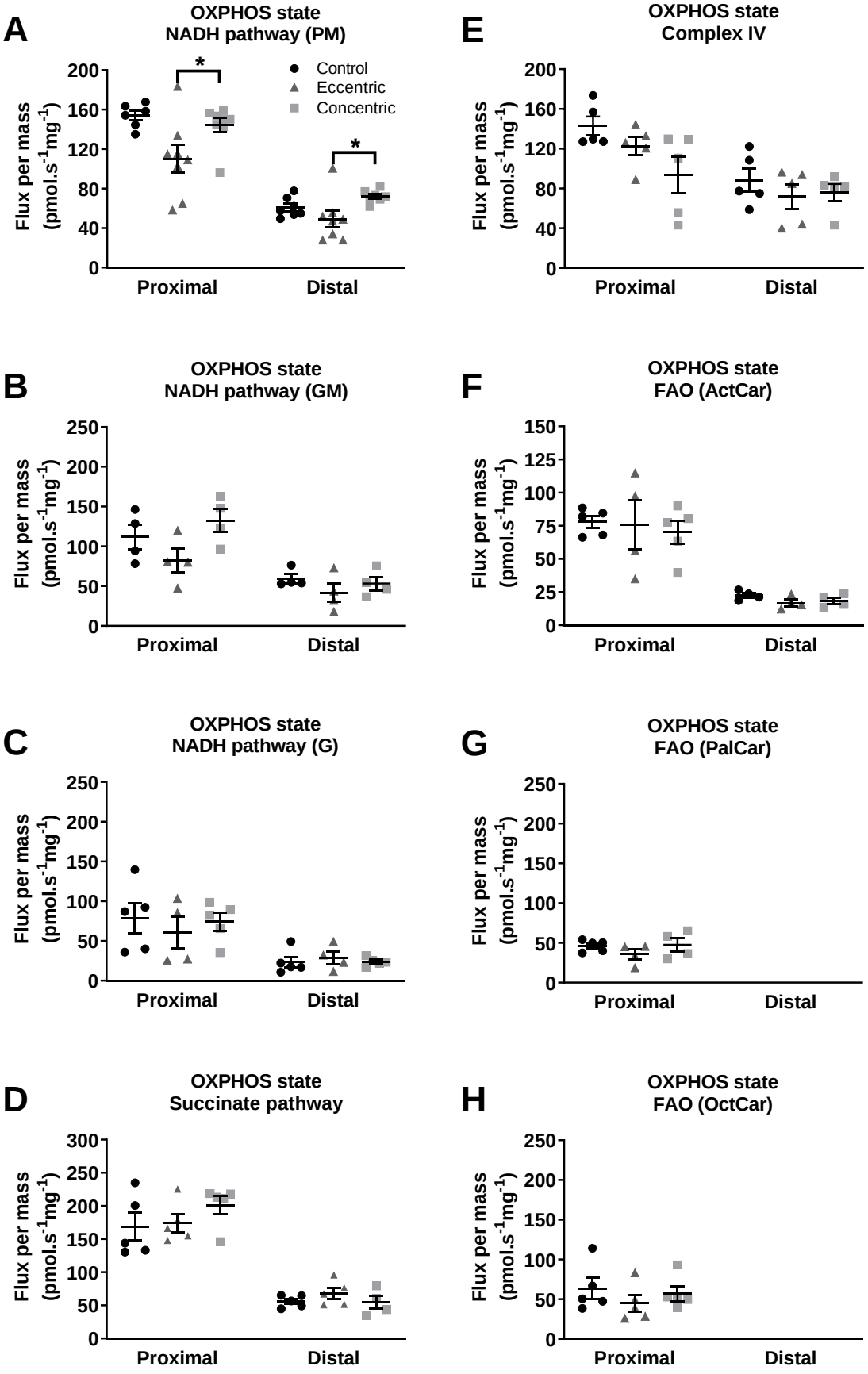
A
OXPHOS state
NADH pathway (PM)
Flux per mass (pmol.s-1mg-1)
Control
Eccentric
Concentric
*
*
Proximal
Distal
B
OXPHOS state
NADH pathway (GM)
C
OXPHOS state
NADH pathway (G)
D
OXPHOS state
Succinate pathway
E
OXPHOS state
Complex IV
F
OXPHOS state
FAO (ActCar)
G
OXPHOS state
FAO (PalCar)
H
OXPHOS state
FAO (OctCar)

FIGURE 2.

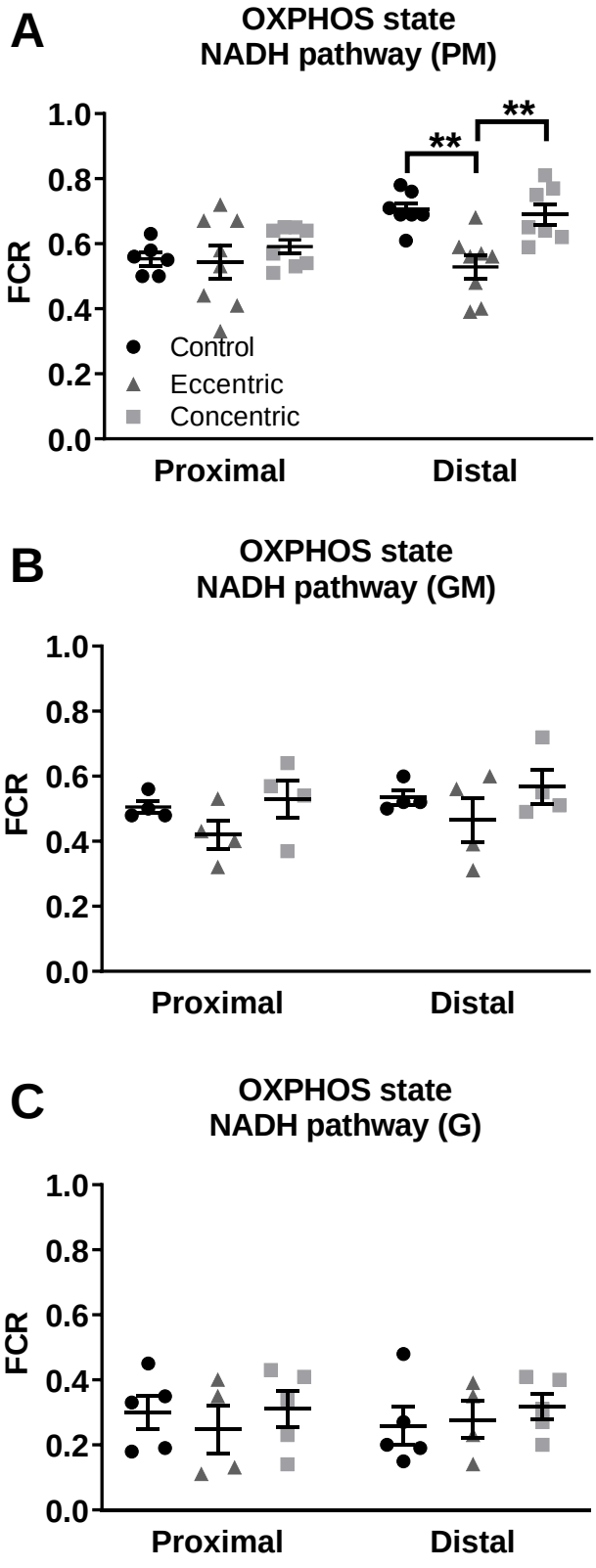
A
OXPHOS state
NADH pathway (PM)
FCR
1.0
0.8
0.6
0.4
0.2
0.0
**
**
Control
Eccentric
Concentric
Proximal
Distal
B
OXPHOS state
NADH pathway (GM)
FCR
1.0
0.8
0.6
0.4
0.2
0.0
Proximal
Distal
C
OXPHOS state
NADH pathway (G)
FCR
1.0
0.8
0.6
0.4
0.2
0.0
Proximal
Distal

FIGURE 3.

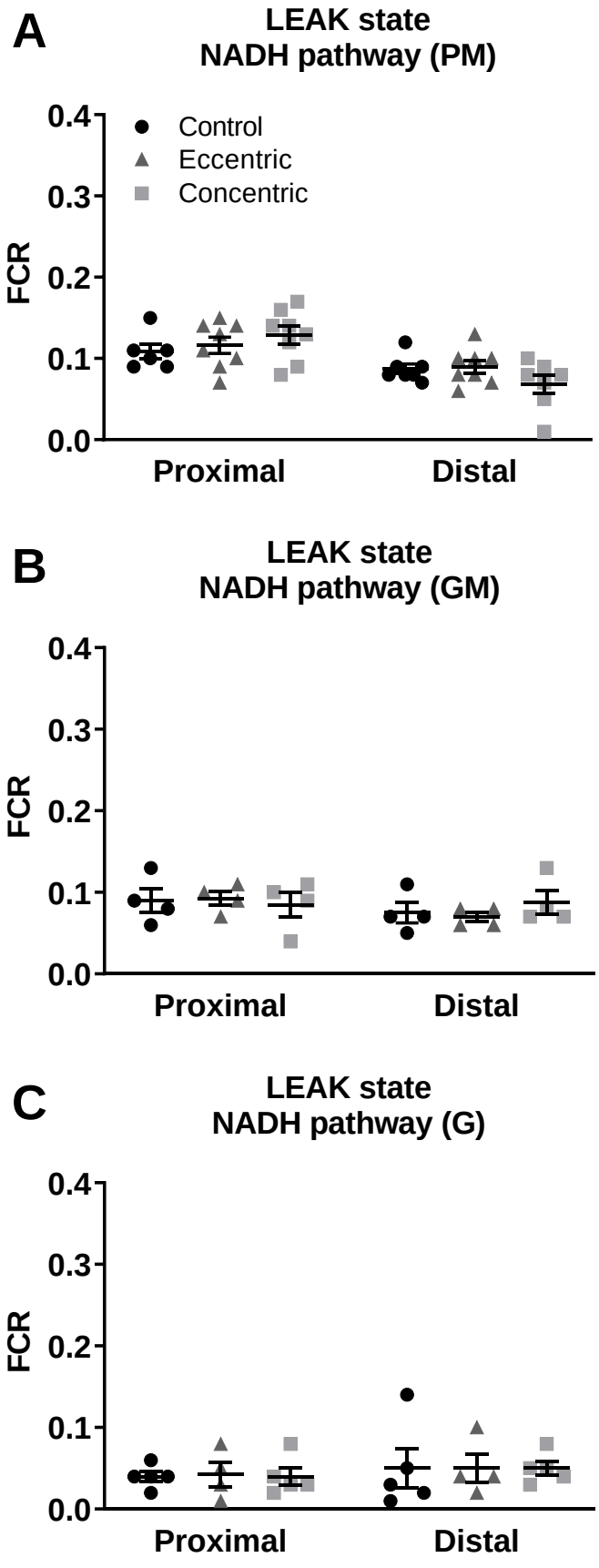
A
LEAK state
NADH pathway (PM)
Control
Eccentric
Concentric
FCR
0.4
0.3
0.2
0.1
0.0
Proximal
Distal
B
LEAK state
NADH pathway (GM)
FCR
0.4
0.3
0.2
0.1
0.0
Proximal
Distal
C
LEAK state
NADH pathway (G)
FCR
0.4
0.3
0.2
0.1
0.0
Proximal
Distal

FIGURE 4.