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RESEARCH ARTICLE

Maternal exercise before and during gestation modifies liver and muscle mitochondria in rat offspring

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ABSTRACT

It is now well established that the intrauterine environment is of major importance for offspring health during later life. Endurance training during pregnancy is associated with positive metabolic adjustments and beneficial effects on the balance between pro-oxidants and antioxidants (redox state) in the offspring. Our hypothesis was that these changes could rely on mitochondrial adaptations in the offspring due to modifications of the fetal environment induced by maternal endurance training. Therefore, we compared the liver and skeletal muscle mitochondrial function and the redox status of young rats whose mothers underwent moderate endurance training (treadmill running) before and during gestation (T) with those of young rats from untrained mothers (C). Our results show a significant reduction in the spontaneous H_2O_2 release by liver and muscle mitochondria in the T versus C offspring (P<0.05). These changes were accompanied by alterations in oxygen consumption. Moreover, the percentage of short-chain fatty acids increased significantly in liver mitochondria from T offspring. This may lead to improvements in the fluidity and the flexibility of the membrane. In plasma, glutathione peroxidase activity and protein oxidation were significantly higher in T offspring than in C offspring (P<0.05). Such changes in plasma could represent an adaptive signal transmitted from mothers to their offspring. We thus demonstrated for the first time, to our knowledge, that it is possible to act on bioenergetic function including alterations of mitochondrial function in offspring by modifying maternal physical activity before and during pregnancy. These changes could be crucial for the future health of the offspring.

KEY WORDS: Endurance training, Intrauterine environment, Oxidative phosphorylation, Offspring

INTRODUCTION

The prenatal environment is widely considered to have a major impact on fetus development (Barker, 1998; Barker and Osmond, 1986; Barker et al., 1989; Raipuria et al., 2015). It can have long-lasting consequences that contribute to the general health of the offspring, as suggested by the concept of the 'developmental origins of health and diseases' (DOHaD) based on epidemiological data (Barker and Osmond, 1986; Hanson and Gluckman, 2014; McMillen and Robinson, 2005).

To enhance their chances of having a successful pregnancy and to improve both their own health and that of their developing child, women are prone to adopt a healthy lifestyle while pregnant (Edvardsson et al., 2011; Funk et al., 2015). Among other factors, regular physical activity during pregnancy is reported to help control body weight and body composition and to improve cardiovascular health (Clapp and Little, 1995). Exercise training is also well recognized to have many positive effects on energy metabolism at both whole-body and cellular levels. Maternal exercise training seems to also be beneficial to the offspring, with improved stress tolerance and advanced neurobehavioural maturation (Melzer et al., 2010; Snapp and Donaldson, 2008). Other benefits from regular exercise during pregnancy for the offspring include lower fat mass at birth and during childhood, as well as improved cognitive characteristics (Clapp, 1996; Dayi et al., 2012). Recent studies in rats have also showed that maternal treadmill or wheel running training during pregnancy modifies insulin sensitivity in adult offspring (Carter et al., 2013; Quiclet et al., 2016). Taken together, these data confirm that maternal regular physical activity could be a good way to improve the fetus environment. However, the mechanisms by which it elicits such metabolic adjustments are unclear. Pregnancy is a physiological state associated with a moderately enhanced oxidative stress related to high metabolic turnover and elevated tissue oxygen requirements (Toescu et al., 2002). Moreover, oxidative stress in pregnant women has been linked to oxidative stress in the fetus (Arguelles et al., 2006). Mitochondria, as intracellular energy-producing 'power houses' and as a significant source of reactive oxygen species (ROS), seem to be at the centre of these modifications. They undergo qualitative and quantitative changes with exercise training as shown by a lower apparent affinity constant (K_m) for pyruvate and palmitoyl Co-A as substrates in permeabilized isolated muscle fibres from offspring of exercising mothers compared with the control group (Quiclet et al., 2017). Such changes could contribute to a better health status (for review, see Granata et al., 2018). Redox status, defined as the balance between oxidants (or pro-oxidants) and antioxidants, also seems to have a fundamental role in the exercise effect, by balancing oxidative damage with an increase in the antioxidant network, although the literature is not unanimous (Radak et al., 2008). One hypothesis to explain how exercise could improve offspring metabolic health is that maternal regular physical activity has an impact on offspring mitochondrial phenotype. Indeed, it has been shown that maternal exercise upregulates genes encoding mitochondrial biogenesis and dynamics along with

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List of abbreviations

C	control untrained group
CS	citrate synthase
DCIP	dichlorophenolindophenol
ETC	electron transport chain
G	glutamate
M	malate
Q_{10}	quinone 10
Q_9	quinone 9
RCR	respiratory control ratio
RET	reverse electron transfer
ROS	reactive oxygen species
S	succinate
T	endurance-trained group

increase in cytochrome *c* oxidase activity in fetal myocardium (Chung et al., 2017). Our aim was therefore to examine whether maternal exercise training could induce functional changes of the mitochondria that could persist in other important tissues of the offspring solicited by exercise. We then studied some aspects of mitochondrial function of isolated skeletal muscle and liver mitochondria of offspring born to endurance-trained dams (T) compared with those born to control dams (C).

MATERIALS AND METHODS

Ethical approval

All experimental procedures were carried out in accordance with European Directive 2010/63/UE. They were reviewed by the Institutional Ethics Committee for Animal Care and Use and authorized by the French Ministry of Research (00174.02 accepted in March 2014).

Animals

Nulliparous 12-week-old female Wistar rats (Charles River Laboratories, Saint Germain-Nuelles, France) were housed three per cage with access to food (A03; SAFE Diets, Augy, France) and water *ad libitum*. The animal facility was on a 12 h:12 h light:dark cycle and maintained at a temperature of 22±2°C.

After a 1 week acclimatization period, female rats were assigned to either a sedentary control (C; n=6) or exercise-trained (T; n=6) group. Body mass and food consumption were monitored once per week during breeding and pregnancy. Trained females were exercised using a motorized treadmill (Bioseb, Vitrolles, France) 5 days per week during the 4 weeks before gestation and during the first 18 days of gestation, whereas female rats from the control group remained sedentary in their cages. The treadmill speed and the duration of the training session were gradually increased during the first 3 weeks of training to reach a speed of 25 m min^{-1} for 60 min with a 10% slope. After 4 weeks of controlled exercise, females from each group were housed with male rats for 1 week for mating. The male rats did not exercise during the study. Vaginal smears were performed each subsequent day until spermatozoa were found, to determine the first day of gestation. The endurance training protocol continued until the 18th day of gestation.

On postnatal day 1, litter sizes were equalized to eight pups to avoid any artifactual effect on pup body mass. As only male pups were considered in the study, to avoid any additional confounding influence of hormonal variation, pups were cross-fostered from other litters from the same group and the same age, to maximize the number of males per litter. Two male pups from each litter were then randomly selected to be used in the study. Mothers from the endurance-trained group did not exercise during nursing and were killed after nursing. Male pups were weighed on postnatal days 1, 7, 14 and 21 and were killed at weaning. The offspring groups were named according to the maternal treatment (control or endurance trained).

In order to verify the efficacy of our moderate-intensity endurance-training protocol, we conducted a pilot study to measure citrate synthase (CS) activity, considered as a good index of mitochondrial biogenesis and a good marker of responses to endurance training (Holloszy and Coyle, 1984), in skeletal muscles and liver of mothers immediately after delivery.

Twelve females following the same training and breeding protocols were weighed and killed and their retroperitoneal, urogenital and mesenteric adipose tissues, as well as right hindlimb plantaris muscles, were collected and weighed. These tissues were frozen in liquid nitrogen and stored at −80°C.

Blood and tissue sampling

At weaning, in the postprandial state, 9 male offspring from each group were weighed and decapitated without anaesthesia. Blood was collected and centrifuged to separate plasma and packed red blood cells before storage at −80°C until further analysis. Retroperitoneal, epididymal, subcutaneous and mesenteric adipose tissues were collected and weighed. Heart, liver and the muscles of both hindlimbs were dissected out. These tissues were frozen in liquid nitrogen and stored at −80°C. Remaining tissues were used to prepare isolated mitochondria as described below. After nursing, 6 mothers from each group were weighed and decapitated without anaesthesia. Retroperitoneal, urogenital, subcutaneous and mesenteric adipose tissues, liver, heart, as well as right hindlimb gastrocnemius, soleus and plantaris muscles were collected and weighed.

Mitochondrial isolation

Mitochondria were isolated from offspring liver according to a standard differential centrifugation procedure (Klingenberg and Slenczka, 1959) and from muscles with adaptations. Muscles from both hindlimbs were minced and transferred into 30 ml of isolation buffer (150 mmol l^{-1} sucrose, 75 mmol l^{-1} KCl, 1 mmol l^{-1} KH_2PO_4, 5 mmol l^{-1} $MgCl_2$, 1 mmol l^{-1} EGTA, 50 mmol l^{-1} Tris-HCl, pH 7.4) supplemented with 0.2% fat-free bovine serum albumin. The suspension was then incubated with 0.2 mg ml^{-1} subtilisin for 1 min and then diluted twice with ice-cold isolation buffer. Liver was dissected out, rinsed and minced in ice-cold isolation buffer (250 mmol l^{-1} sucrose, 50 mmol l^{-1} Tris-HCl, 1 mmol l^{-1} EGTA, pH 7.4). Liver and muscle preparations were then (separately) homogenized using a motor-driven glass–Teflon pestle homogenizer. Nuclei and cell debris were removed by centrifugation (800 ***g***, 10 min, 4°C). Mitochondria were collected from the supernatants by centrifugation (twice at 8000 ***g***, 10 min, 4°C). Each mitochondrial pellet was resuspended in 0.5 ml isolation buffer and kept on ice. Mitochondrial protein content was determined using the bicinchoninic acid assay (Pierce) using bovine serum albumin (BSA) as a standard.

Oxygen consumption

Rates of oxygen consumption of isolated mitochondria were measured using a Clark-type O_2 electrode (Oxygraph, Hansatech Instruments). Muscle (0.2 mg ml^{-1}) or liver (1 mg ml^{-1}) mitochondria were incubated at 30°C in a 1 ml chamber filled with respiration buffer containing 125 mmol l^{-1} KCl, 5 mmol l^{-1} inorganic phosphate, 20 mmol l^{-1} Tris-HCl, 0.1 mmol l^{-1} EGTA, 0.1% fat-free BSA (pH 7.2). The suspension was constantly stirred with a built-in electromagnetic stirrer and bar flea. Measurements

were carried out in the presence of 5 mmol l^{-1} glutamate/2.5 mmol l^{-1} malate (G/M) as substrates of complex I, 5 mmol l^{-1} succinate (S) as a substrate of complex II, or 5 mmol l^{-1} succinate plus 5 mmol l^{-1} glutamate/2.5 mmol l^{-1} malate (S+G/M), after the addition of 1 mmol l^{-1} ADP (state 3), followed by the addition of 0.25 mg ml^{-1} oligomycin (state 4). Respiratory control ratio (RCR) was obtained by dividing oxygen consumption in state 3 by oxygen consumption in state 4.

Mitochondrial H_2O_2 release

Mitochondrial production of ROS was estimated by measurement of H_2O_2 release based on the increase in fluorescence due to enzymatic oxidation of Amplex Red® (excitation at 560 nm, emission at 584 nm) by H_2O_2 in the presence of horseradish peroxidase. Muscle or liver mitochondria (0.2 mg ml^{-1}) were incubated at 30°C in a stirred 1 ml chamber in the respiration medium in the presence of 6 U ml^{-1} of horseradish peroxidase and 1 µmol l^{-1} Amplex Red®. Measurements were carried out with: (i) 5 mmol l^{-1} succinate (S) to provide electrons to the electron transport chain (ETC) from complex II and (ii) 5 mmol l^{-1} succinate plus 5 mmol l^{-1} glutamate/2.5 mmol l^{-1} malate (S+G/M) to provide electrons to the ETC from both complex I and complex II, followed by successive additions of (iii) 2 µmol l^{-1} rotenone (specific complex I inhibitor) and (iv) 1.5 µmol l^{-1} antimycin A (specific complex III inhibitor).

Determination of enzymatic activity

Enzymatic activity of complexes of the ETC in liver and muscle mitochondria

Specific activity of respiratory chain complexes I, II and III was determined spectrophotometrically and expressed as µmoles of reduced or oxidized substrate per minute per mg of mitochondrial protein.

Complex I (rotenone-sensitive NADH-ubiquinone oxidoreductase) activity was assayed using 100 µmol l^{-1} decylubiquinone as the electron acceptor and 200 µmol l^{-1} NADH as the donor, in a 10 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer, pH 7.5, containing 3.75 mg ml^{-1} BSA, 2 mmol l^{-1} KCN and 7.5 µmol l^{-1} antimycin A. Oxidation of NADH was then measured at 340 nm, before and after the addition of 4 µmol l^{-1} rotenone to allow calculation of the rotenone-sensitive specific activity, which is characteristic of complex I.

Complex II (succinate-ubiquinone reductase) activity was determined by measuring the decrease in absorbance due to the reduction of dichloroindophenol (DCIP) at 600 nm. The measurement was performed in 50 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer, pH 7.5, in the presence of 100 µmol l^{-1} decylubiquinone as substrate, 100 µmol l^{-1} DCIP, 20 mmol l^{-1} succinate, 2 µmol l^{-1} rotenone and 2 mmol l^{-1} KCN.

Complex III (ubiquinol cytochrome *c* reductase) activity was determined by measuring the reduction of cytochrome *c* when decylubiquinol was used as substrate and complexes I and IV were blocked by specific inhibitors. The change in absorbance at 550 nm was first measured at 30°C for 1 min in the presence of 90.7 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer, pH 7.4, 50 µmol l^{-1} EDTA, 1 mg ml^{-1} BSA, 1 mmol l^{-1} KCN, 100 µmol l^{-1} oxidized cytochrome *c* and 0.11 mmol l^{-1} decylubiquinol in order to evaluate total activity. The non-specific activity was then measured after addition of 5 µg ml^{-1} antimycin A for 2 min. Complex III-specific activity was then obtained by subtraction.

Complex IV (cytochrome *c* oxidase) activity was assayed by oxygraphy on freshly isolated liver and muscle mitochondria in respiration buffer (see above) using 0.5 mmol l^{-1} TMPD/2.5 mmol l^{-1} ascorbate as electron donor/acceptor.

CS activity

Plantaris muscle or liver samples (50 mg) were homogenized at 4°C in 450 µl of 100 mmol l^{-1} KH_2PO_4, pH 7.4. The homogenates were centrifuged (1500 ***g***, 5 min, 4°C), and the resulting supernatant was collected and stored at −80°C until assays. CS activity was assessed according to Singh et al. (1970) and expressed as µmol DTNB min^{-1} g^{-1} of wet tissue.

Glutathione peroxidase (GPX) activity

A portion of frozen liver (100 mg) was homogenized with a glass–Teflon potter at 4°C, in 100 mmol l^{-1} KH_2PO_4, 1 mmol l^{-1} DTT and 2 mmol l^{-1} EDTA, pH 7.4. After centrifugation (3000 ***g***, 5 min), the supernatant was used for enzymatic assays. GPX activity in supernatant and in plasma was evaluated by the modified method of Gunzler et al. (1974) using terbutyl hydroperoxide (Sigma-Aldrich, Saint Quentin Fallavier, France) as a substrate.

Other biochemical assays

Quantification of fatty acid esters was performed by gas chromatography coupled to mass spectroscopy. Samples from liver mitochondria containing internal standards were saponified in 0.6 mol l^{-1} ethanolic potassium hydroxide. Fatty acids were further extracted, derivatized and resuspended in 100 µl hexane, and 1 µl was used for the determination of fatty acid composition (Viens et al., 1996). Cytochrome content of the respiratory chain was measured in liver mitochondria by comparison of the spectra of fully oxidized cytochromes (using potassium ferricyanide) with that of fully reduced cytochromes (using sodium dithionite). Absorbance values were used to calculate the amount of cytochrome (Williams, 1964). Quinones 9 (Q_9) and 10 (Q_{10}) were measured on powdered frozen tissues, liver mitochondria and plasma. After solubilization and extraction in 2-propanol, Q_9 and Q_{10} were detected by reverse-phase HPLC with electrochemical detection on the same run (Galinier et al., 2004).

Plasma and liver protein oxidation level was evaluated spectrophotometrically at 415 nm as the disappearance of protein thiol groups (Faure and Laffond, 1995). Standards and samples (20 µl) were incubated in 50 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer, 100 mmol l^{-1} EDTA, pH 8, and 10 mmol l^{-1} bis-5,5′-dithio-bis(2-nitrobenzoic acid).

Statistical analyses

All data are expressed as means±s.e.m. One-way analysis of variance (ANOVA) was used to determine the global effects of endurance training of mothers on their offspring. When appropriate, differences between groups were tested with Holm–Šidák *post hoc* tests. Statistical significance was accepted at $P<0.05$. Kruskal–Wallis tests were applied when values were not normally distributed (Sigma Plot®).

RESULTS

Effects of endurance training on mothers

Skeletal muscle and liver CS activity measured immediately after delivery in the pilot study were higher ($P<0.05$) in endurance-trained mothers than in controls (Table 1). Endurance training before and during gestation had no effect on maternal mass gain (Table 1) or on maternal food intake during the last week of gestation (Table 1). The sum of the mass of the three visceral white adipose tissues (mesenteric, retroperitoneal and urogenital) or the

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Table 1. Characteristics of untrained (control, C) and endurance-trained (T) mothers

	C	T
CS activity (pilot study)		
Liver (µmol DTNB min^{-1} g^{-1} wet tissue)	73±3	83±4*
Muscle (µmol DTNB min^{-1} g^{-1} wet tissue)	240±20	325±16*
Body mass (g)		
Before gestation	245±5	263±14
Post-partum	261±5	279±9
Mass gain during gestation (g)	16±1	16±8
Food intake during the last week of gestation (g 100 g^{-1} body mass)	53±6	62±16
Organ mass (g 100 g^{-1} body mass)		
Liver	4.79±0.14	4.80±0.29
Heart	0.287±0.006	0.285±0.009
Visceral fat	2.72±0.36	2.03±0.28
Subcutaneous fat	0.83±0.11	0.99±0.08
Muscle	0.60±0.02	0.54±0.02

CS, citrate synthase. Values are means±s.e.m. of n=6 rats per group.
*Significantly different from the control group (P<0.05).
Visceral fat mass was calculated as the sum of mesenteric, retroperitoneal and urogenital adipose tissue mass. Muscle mass was calculated as the sum of gastrocnemius, plantaris and soleus muscle mass. Mass gain during gestation corresponds to the difference in mass at delivery and at mating. CS activity was determined in a pilot study designated to verify the efficacy of the training protocol used. Experimental conditions are described in Materials and Methods.

mass of the subcutaneous adipose tissue was not altered by endurance training (Table 1). Endurance training had no effect on heart, liver or muscle mass either (Table 1). Litters were similar for endurance-trained and control groups of mothers, with 11 pups per litter and no significant difference in the male sex ratio (63% versus 45%, respectively, non-significant).

Effects of endurance training of mothers on their offspring

Body composition

Offspring from endurance-trained mothers had a significantly higher body mass than those from control mothers from postnatal day 7 to postnatal day 21 (Fig. 1A), a higher heart relative mass (Fig. 1B) but a similar visceral adipose tissue relative mass (Fig. 1C) and subcutaneous adipose tissue relative mass (0.438±0.044 g 100 g^{-1} body mass in C offspring versus 0.516±0.031 g 100 g^{-1} body mass in T offspring). Additionally, endurance training of mothers had no effect on liver or muscle CS activity (Table 2).

Mitochondrial functioning

ROS production

With S or S+G/M as substrates, mitochondrial H_2O_2 release was significantly lower in offspring from endurance-trained mothers than in those from control mothers for both liver (Fig. 2A, P<0.05) and muscle (Fig. 2B, P<0.05). After rotenone addition, mitochondrial H_2O_2 release was similar in liver (Fig. 2A) and in muscle (Fig. 2B) for the two groups of offspring. Antimycin A addition increased H_2O_2 release compared with that measured after rotenone addition, but without any significant difference between groups for both liver (Fig. 2A) and muscle (Fig. 2B).

Oxygen consumption

There was a significant decrease in state 3 (+ADP) oxygen consumption with G/M, S and S+G/M (P<0.05) as substrates in liver mitochondria of offspring from endurance-trained mothers compared with those from control mothers (Fig. 3A). When S was used as a substrate, state 3 oxygen consumption by muscle mitochondria from offspring of endurance-trained mothers was significantly lower than that from offspring of control mothers (Fig. 3B). This difference was not seen when S+G/M was used as a substrate (Fig. 3B). RCR was significantly lower when S or S+G/M was used as a substrate in liver and in muscle mitochondria from offspring of endurance-trained mothers compared to those of control mothers (insets in Fig. 3A,B).

Respiratory complex activities

In liver, complex I and III activities were not different between groups (Table 2). However, complex II activity was significantly lower (P<0.05) while complex IV activity was higher (P<0.05) in offspring from endurance-trained mothers than in those from control mothers. In muscle, complex I activity between the two groups was similar (Table 2) but complex II (P<0.05) and III (P<0.05) activities were lower in offspring from endurance-trained mothers than in those from control mothers, while complex IV activity was higher (P<0.05) (Table 2).

Mitochondrial composition

Cytochromes

There was a higher (P<0.05) cytochrome a+a_3 content in liver mitochondria of offspring from endurance-trained mothers compared with those from control mothers (Table 3) while cytochrome b, c+c_1 and c content was not different between groups.

Quinones

There was a significant increase in reduced Q_9 (P<0.05) and Q_{10} (P<0.05) in liver of offspring from endurance-trained mothers compared with those from sedentary mothers (Table 3). Oxidized Q_9 levels (oxidized Q_{10} was not detectable) and reduced/oxidized Q_9 ratio were not significantly different in offspring from endurance-trained mothers. Interestingly, the higher reduced Q_9 and reduced Q_{10} content in the T group was accompanied by an increase in quinone pools (total Q_9+Q_{10}) and in α-tocopherol content (Table 3).

Fatty acid composition of liver mitochondria

Endurance training of the mothers was associated with significant changes in liver mitochondrial fatty acid content and composition in the offspring. Indeed, there was an increase (P<0.05) in the percentage of short-chain fatty acids and a decrease (P<0.05) in the percentage of long-chain fatty acids in offspring from endurance-trained mothers compared with those from control mothers (Table 3). The proportion of unsaturated fatty acids was lower (P<0.05) in offspring from the endurance-trained group than in those from the control group, while the proportion of saturated fatty acids was higher (P<0.05). Total n-3 fatty acids and n-3/n-6 ratio were also significantly higher in offspring from endurance-trained mothers than in offspring from control mothers (Table 3).

Redox status

The blood and liver redox status of offspring from endurance-trained mothers was altered compared with that of offspring from sedentary mothers (Fig. 4). There was a higher GPX activity in blood (Fig. 4A) and in liver (Fig. 4B) associated with an increase in oxidized proteins in blood (Fig. 4A), as indicated by a significantly lower concentration of thiols in the offspring of endurance-trained mothers.

DISCUSSION

This study shows that maternal endurance training can have effects on liver and muscle mitochondrial function of offspring. More

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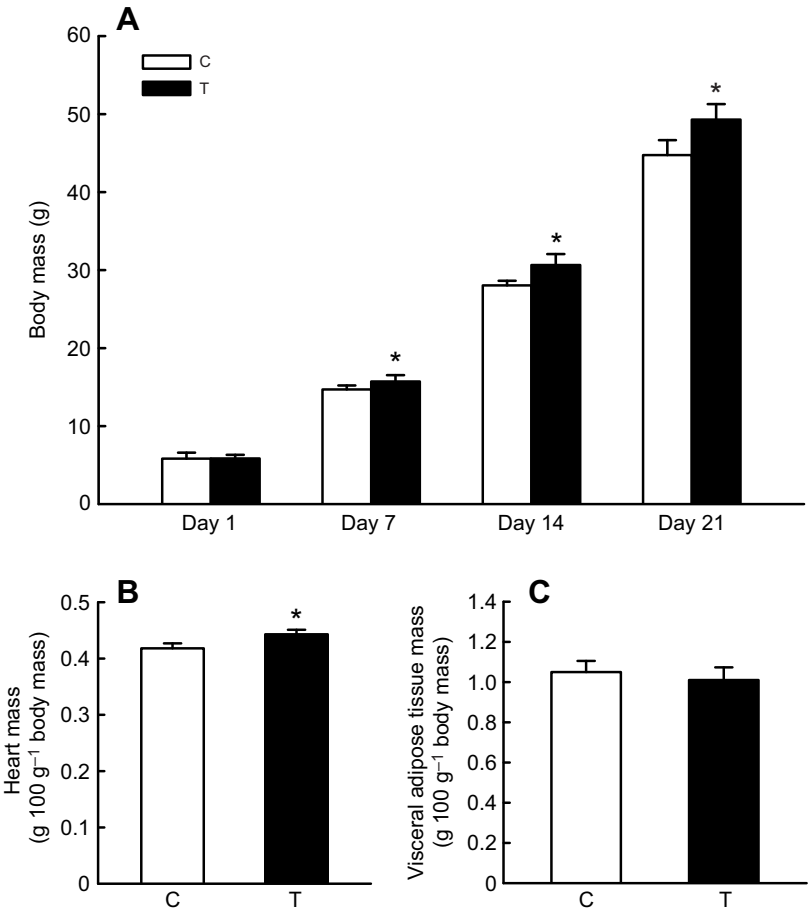

Fig. 1. Characteristics of offspring from untrained (control, C) and endurance-trained (T) mothers. (A) Body mass from postnatal day 1 to day 21 (weaning). (B) Relative heart mass at day 21. (C) Relative visceral adipose tissue mass. Values are means±s.e.m. of *n*=9 rats per group. *Significantly different from the control group (*P*<0.05). Experimental conditions are described in Materials and Methods.

specifically, such training of the mothers is associated with lower mitochondrial H_2O_2 release and alterations of mitochondrial membrane composition in the offspring. However, little is known about the transmittance of these endurance effects to the offspring.

To study the effect of regular maternal exercise on offspring, we chose a model of treadmill endurance training suitable during the gestation of already active, fit females. Studies on rodents using voluntary exercise with running wheels showed that the running distance decreased dramatically in the last week of gestation (Eclarinal et al., 2016). Treadmill exercise avoids any change in the training parameters and, in particular, any reduction in exercise intensity and duration until day 18 of gestation. Female rats were trained for 4 weeks before mating, as such a duration is usually sufficient to observe the first signs of adaptation to chronic endurance exercise (Wisløff et al., 2001). To maximize the effect of that training period, we also chose a sufficiently high intensity, based on parameters classically found in the literature (Bedford et al., 1979). Additional data from other studies in our laboratory showed that the speed and slope of the treadmill that we used correspond to an intensity of about 55% of the maximal aerobic speed (data not shown). To objectively assess the effectiveness of the endurance-training protocol, we first conducted a pilot study on female rats using the same parameters to establish that the endurance training imposed before and during gestation (i) induces adaptations and (ii) does not disturb the gestation or does not endanger the life of the mother and offspring. The higher maximal activity of CS, considered as a classical marker of skeletal muscle adaptation to endurance training (Carter and Hood, 2012; Holloszy and Coyle, 1984; Vigelso et al., 2014), found in liver and muscle of the endurance-trained mothers in the pilot study indicates that our training protocol was indeed effective. Moreover, besides these effects on the mitochondria, this training protocol had no effect on maternal body mass, food intake or body composition.

Some authors have reported that voluntary and controlled moderate-intensity exercise during gestation has no negative consequences on dams and litters (Carter et al., 2013; Platt et al., 2013). The delivery of oxygen and substrate to the maternal–fetal interphase is the major maternal environmental stimulus which

Table 2. Enzymatic activity of citrate synthase and electron transport chain complexes in liver and muscle of offspring from untrained (C) and endurance-trained (T) mothers

	C	T
Liver		
CS (μmol DTNB min^{-1} g^{-1} wet tissue)	57±4	56±2
C-I (μmol NADH min^{-1} mg^{-1} protein)	29±3	29±3
C-II (μmol DCIP min^{-1} mg^{-1} protein)	241±6	210±3*
C-III (μmol cytochrome *c* min^{-1} mg^{-1} protein)	484±17	484±24
C-IV (nmol O_2 min^{-1} mg^{-1} protein)	207±22	284±13*
Muscle		
CS (μmol DTNB min^{-1} g^{-1} wet tissue)	228±14	251±10
C-I (μmol NADH min^{-1} mg^{-1} protein)	54±6	51±5
C-II (μmol DCIP min^{-1} mg^{-1} protein)	549±48	340±25*
C-III (μmol cytochrome *c* min^{-1} mg^{-1} protein)	3046±96	2449±128*
C-IV (nmol O_2 min^{-1} mg^{-1} protein)	708±47	869±27*

C-I–IV, complex I–IV. Values are means±s.e.m. of *n*=8 rats per group.
*Significantly different from the control group (*P*<0.05).
Experimental conditions are described in Materials and Methods.

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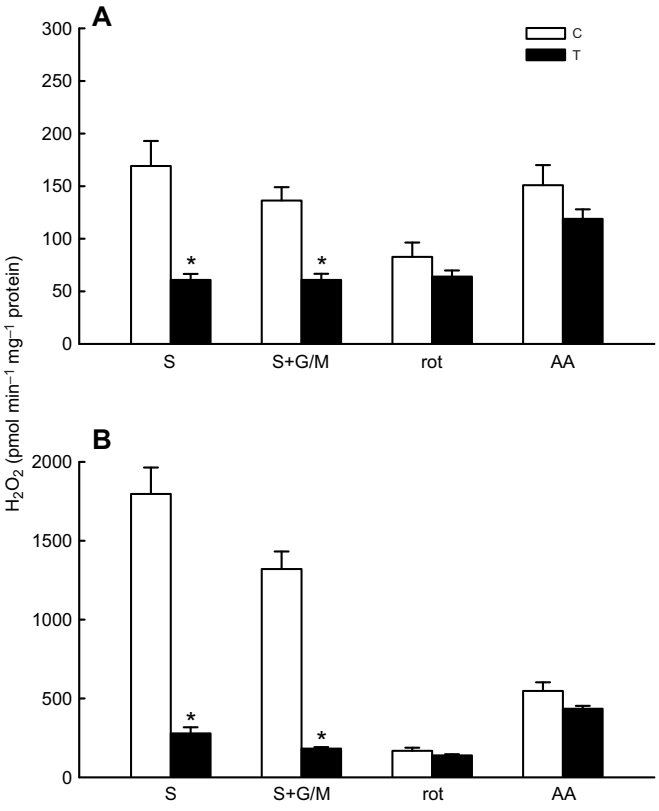

Fig. 2. Mitochondrial H_2O_2 release in offspring from untrained (C) and endurance-trained (T) mothers. (A) Liver. (B) Muscle. Substrates: S, succinate; G/M, glutamate/malate; S+G/M, succinate+glutamate/malate. Inhibitors: rot, rotenone; AA, antimycin A. Values are means±s.e.m. of n=8 rats per group. *Significantly different from the control group (P<0.05). Experimental conditions are described in Materials and Methods.

either up- or down-regulates feto-placental growth. In general, exercise in early and mid-pregnancy stimulates placental growth while the relative amount of exercise in late pregnancy determines its effect on late fetal growth (Clapp, 2006). Our training protocol appears to be safe for the mother and the litter as no differences were found in sex ratio, number or birth mass of pups, suggesting that moderate-intensity endurance training before and during gestation does not affect the delivery of nutrients to the fetus (Clapp, 2006; Hopkins et al., 2010). However, the higher body mass as early as day 7 and until day 21 in offspring born to endurance-trained mothers compared with those from sedentary mothers, without any significant difference in either visceral fat mass or the relative mass of the subcutaneous adipose tissue, suggests that this increase is due to a higher lean body mass. Such an increase is supported by the higher cardiac mass in the offspring from endurance-trained mothers as heart mass has been reported to be correlated with lean body mass (Daniels et al., 1995). This is supported by the literature showing that moderate maternal physical activity during gestation is associated with an increase in placental size and function and higher blood volume (Clapp, 2006), which may explain the increase in lean mass (Bisson et al., 2017). Moreover, endurance training during pregnancy is known to improve insulin sensitivity and to reduce circulating insulin levels in mothers (Embaby et al., 2016). Although we did not measure circulating growth factor or insulin levels in our study, others have reported increased body mass and plasma IGF1 concentrations in fetuses from mothers that exercised prior to and during pregnancy compared with those from sedentary mothers (Mangwiro et al., 2018). It is possible that such hormonal modifications due to training of the mothers occurred in our study, leading to an increase in the lean mass of the offspring. Other studies have also shown that endurance training during gestation and lactation can lead to an increase in protein concentration in the milk and thus act on the lean mass of the offspring (Matsuno et al., 1999). In our study, as the mothers were not trained during lactation, it is unlikely that the milk composition was altered between groups.

To investigate the effects of maternal chronic exercise on the redox balance of offspring, we first studied mitochondrial ROS production levels in liver and skeletal muscle with a focus on succinate and glutamate/malate as substrates to dissect the respective involvement of complex II and complex I as sources of electrons for the ETC. The lower ROS production in the endurance-trained group in the presence of succinate alone or succinate+glutamate/malate in the liver and in the muscle suggests that maternal training can act on the redox balance. When mitochondria were respiring with succinate, the addition of glutamate/malate (S+G/M) and then of rotenone led to a significant reduction in ROS production that is characteristic of the production of ROS with succinate (or S+G/M) by reverse electron transfer (RET-ROS production) through complex I (Batandier et al., 2006). While this RET-ROS production through complex I was apparent in the control group, successive additions of glutamate/malate (S+G/M) and then of rotenone had no effect on ROS production in the endurance-trained group, suggesting that endurance training of the mother exerts a specific effect on RET-ROS production in the offspring. Such an effect could be of significant importance to future offspring health as RET-ROS production has been suggested to be involved in the pathophysiology of cellular functioning, such as alterations in the signalling pathway for immune, inflammatory reactions or in cellular oxidative damage eventually leading to cell death (Dan Dunn et al., 2015; Scialò et al., 2017). Addition of antimycin A is known to have two main effects on mitochondrial ROS production. First, antimycin A suppresses RET by collapsing the membrane potential (thus suppressing RET-ROS production by complex I in the presence of S or S+G/M). Second, antimycin A induces ROS production in complex III by blocking electron transfer in the quinone cycle. In our study, addition of antimycin A reduced the differences between the endurance-trained group and the control group both in liver and in muscle, suggesting that the amount of ROS produced by complex III was similar in the two groups. Thus, differences in ROS production observed in the offspring of the trained mothers would therefore be mainly located at the complex I level, through RET, reinforcing the interest in regular exercise as an important tool for health.

To determine whether these decreases in ROS production with maternal training were related to the function of the ETC, we also measured mitochondrial oxygen consumption and enzymatic activities of isolated ETC complexes. We found that liver mitochondria from the endurance-trained group had a lower oxygen consumption in coupled state 3 compared with the control group, whatever the substrate (G/M, S or S+G/M), leading to significant differences in RCR values for S and S+G/M. This functional measurement of respiration was confirmed by a higher level of reduced quinones in the ETC in the endurance-trained group. This lower mitochondrial respiration in the endurance-trained group seems not to be due to a decrease in the activity of complex I or III, as they are similar in the two groups. However, these differences in oxygen consumption could mainly rely on complex II, as suggested by its lower activity in liver mitochondria of the endurance-trained group. This result is consistent with the

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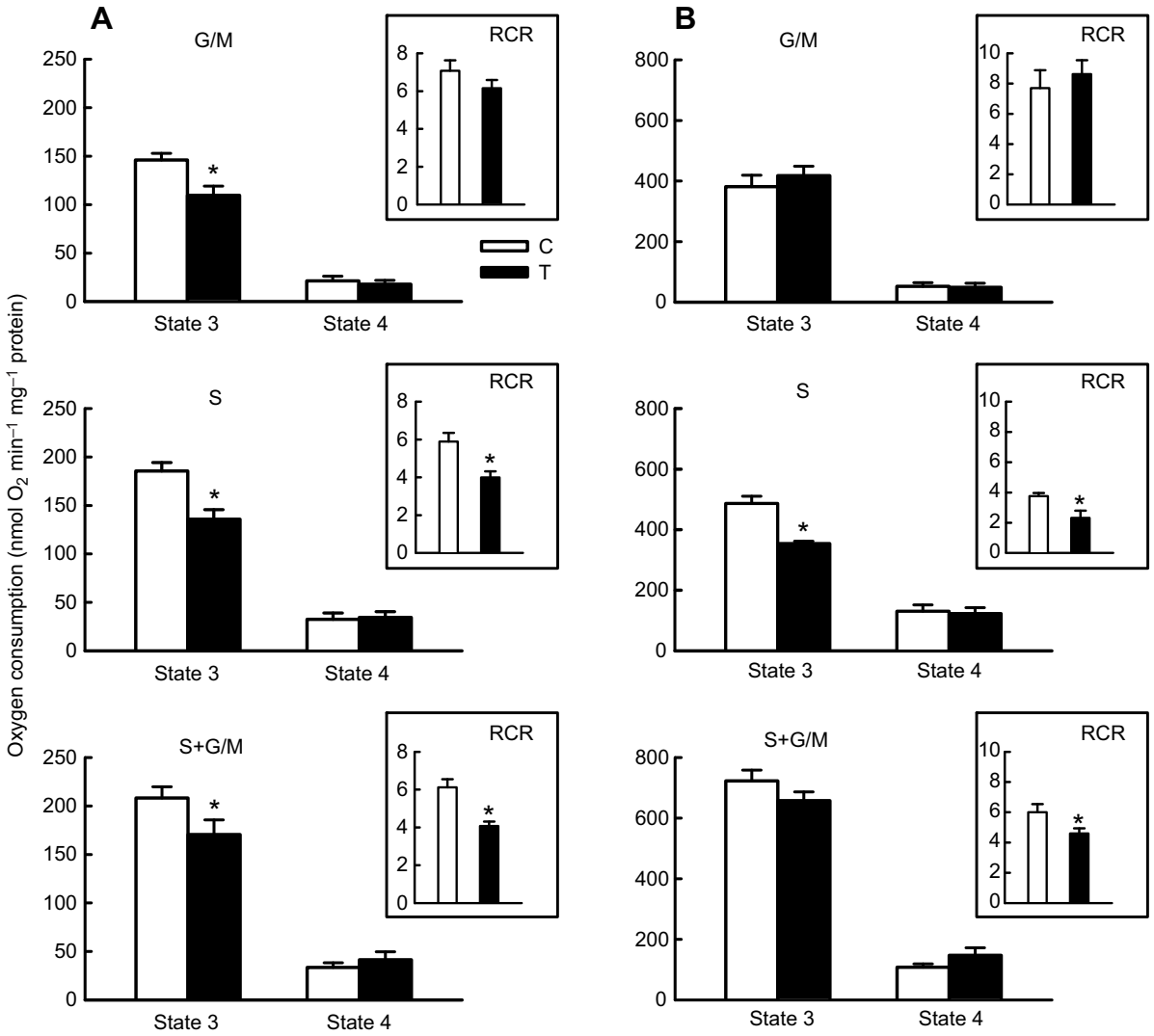

Fig. 3. Mitochondrial oxygen consumption with different substrates in offspring from untrained (C) and endurance-trained (T) mothers. (A) Liver. (B) Muscle. Respiratory control ratio (RCR) was obtained by dividing state 3 respiration by state 4 respiration in each condition (insets). Substrates: S, succinate; G/M, glutamate/malate; S+G/M, succinate+glutamate/malate. Values are means±s.e.m. of n=8 rats per group. *Significantly different from the control group (P<0.05). Experimental conditions are described in Materials and Methods.

lower H_2O_2 production with succinate as a substrate in the endurance-trained group (see above). Maternal exercise appears to have the same effect in muscle: the activity of complex II was significantly lower and the mitochondrial respiration showed a significant decrease with succinate in the endurance-trained group. Consistently, RCR values determined with S and S+G/M as substrates were significantly lower in the endurance-trained group.

The greater complex IV activity in the endurance-trained group both in liver and in muscle mitochondria is consistent with the significant increase in the amount of cytochrome a+a_3 (constitutive of complex IV) reported in liver mitochondria. However, it should be noted that the amount of cytochrome c, which can act as an antioxidant, was similar in the two groups. Knowing that complex IV is not a limiting step in the ETC, to date, we have no explanation for these observations.

Essential indications on the redox status of the offspring were further explored through two major parameters of antioxidant defence (i.e. GPX and oxidized thiols). The higher GPX activity in plasma and liver along with the significantly lower plasma thiol content in the endurance-trained group suggest a reinforced antioxidant defence with training of the mothers. This is confirmed by the higher liver mitochondrial content in α-tocopherol, considered as the most potent radical-scavenging lipophilic agent as it acts as a direct electron scavenger (Lass and Sohal, 1998; Lobo et al., 2010). In biological membranes, the α-tocopherol/phospholipid ratio is low because of the latter being abundant and highly susceptible to oxidative damage. An increase in the level of α-tocopherol would then have beneficial effects either as a peroxyl radical scavenger (Traber and Atkinson, 2007) or to modulate a number of cell functions (Azzi, 2007; Rimbach et al., 2002). The antioxidant defence thus appeared to be globally increased. Unfortunately, similar measurements in the muscle could not be achieved because of the small amount of material available.

We also present novel data on the alteration of the fatty acid profile of liver mitochondria in offspring born to endurance-trained mothers. Indeed, the percentage of short-chain fatty acids increased significantly, while the proportion of long-chain fatty acids was significantly lower in liver mitochondria of offspring from the endurance-trained mothers. These changes may have a beneficial effect as short-chain fatty acids are more resistant to free radical

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Table 3. Liver mitochondria composition in offspring from untrained (C) and endurance-trained (T) mothers

	C	T
Cytochrome content of liver mitochondria		
Cytochrome a+a_3 (pmol mg^{-1} protein)	131±53	261±46*
Cytochrome b (pmol mg^{-1} protein)	190±28	193±17
Cytochrome c+c_1 (pmol mg^{-1} protein)	208±64	307±64
Cytochrome c (pmol mg^{-1} protein)	126±20	120±30
ETC composition in liver		
α-Tocopherol (nmol g^{-1} liver)	8±1	13±1*
Reduced Q_9 (nmol g^{-1} liver)	51±2	58±1*
Reduced Q_{10} (nmol g^{-1} liver)	9.0±0.5	11.0±1.0*
Oxidized Q_9 (nmol g^{-1} liver)	12±1	12±1
Reduced/oxidized Q_9 ratio	4.0±0.5	5.0±0.5
Total Q_9+Q_{10} (nmol g^{-1} liver)	72±2	83±1*
Fatty acid composition of isolated liver mitochondria		
Short chain FA 12:0 (%)	0.045±0.004	0.14±0.009*
Long chain FA 14:0 (%)	99.950±0.004	99.860±0.009*
Very long chain FA 26:0 (%)	0.001±8.55E−5	0.001±4.36E−5
Total UFA (%)	56.0±0.5	53.0±0.5*
Total SFA (%)	44.0±0.5	47.0±0.5*
Total n-3 FA (%)	12.6±0.4	14.1±0.3*
Total n-6 FA (%)	36.1±0.4	32.9±0.4*
(n-3)/(n-6) ratio	0.35±0.01	0.43±0.01*
Grand total (ng g^{-1} protein)	0.13±0.01	0.14±0.01

ETC, electron transport chain; UFA, unsaturated fatty acids; SFA, saturated fatty acids. Values are means±s.e.m. of n=8 rats per group.
*Significantly different from the control group (P<0.05).
Experimental conditions are described in Materials and Methods.

attack or lipid peroxidation compared with long-chain fatty acids (Hulbert et al., 2014). This may lead to an improvement in the fluidity and flexibility of the cellular membranes, helping to maintain their biological role. Additionally, the higher n-3/n-6 ratio in the liver mitochondria of offspring from endurance-trained mothers, due to an increase in n-3 fatty acids and a decrease in n-6 fatty acids, could contribute to protection of the offspring liver mitochondria against inflammatory processes (Wall et al., 2010). Mitochondria are no longer considered as single entities but as parts of a larger mitochondrial network that constantly fuse and divide to maintain normal cellular functions (Calvani et al., 2013). When this delicate balance between division and fusion is lost, mitochondrial function, metabolism and signalling are altered. Pathological conditions including cancer, neurodegeneration and metabolic disorders as well as ageing have been associated with alterations in the balance between fusion and division. Although many studies have sought to understand the dynamic nature of this process, the complete molecular mechanisms underlying it remain unclear (Roy et al., 2015). The lipid composition of the mitochondrial membranes that provides fluidity is probably of major importance in these processes (Aufschnaiter et al., 2017). This is consistent with our findings about the reduction in the length of the fatty acid chains with maternal endurance training as a favourable parameter for the mitochondrial dynamics through a greater mitochondrial membrane fluidity. All these converging elements could represent an adaptive signal of the offspring to the consequences of maternal endurance training.

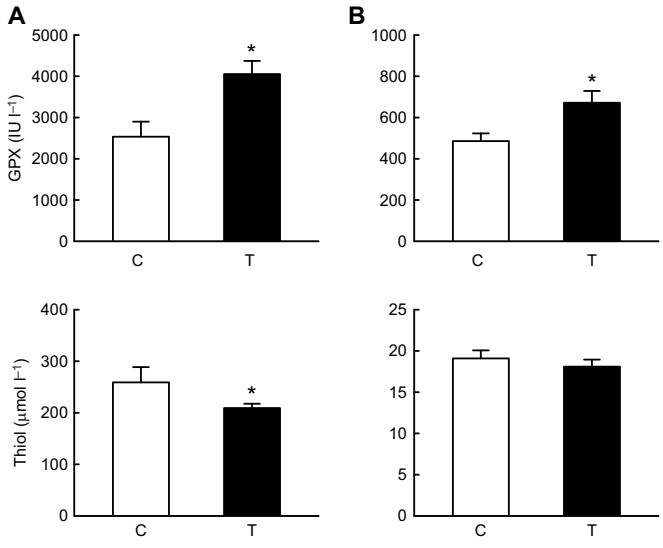

Fig. 4. Glutathione peroxidase (GPX**) activity and thiol content in offspring** from untrained (C) and **endurance-**trained (T) mothers. (A) Plasma. (B) Liver. Values are means±s.e.m. of n=5–9 rats per group. *Significantly different from the control group (P<0.05). Experimental conditions are described in Materials and Methods.

Conclusions

The objective of this study was to determine whether endurance training of the mother during gestation could affect the mitochondrial function of their offspring. We have shown decreased ROS production in liver and muscle mitochondria, with succinate alone or with succinate plus glutamate/malate, in offspring of endurance-trained mothers. This decrease was localized in complex I through the RET. This reduction in RET-ROS production could be linked to a decrease in mitochondrial respiration with succinate (complex II). Moreover, beyond the effect on function, the structure of the mitochondria of the offspring seems also to be modified by maternal endurance training. Taken together with increased protein damage and higher GPX activity in plasma, these findings suggest that maternal endurance training is responsible for redox signal adaptations; the mechanisms involved must be clarified in future studies. Moreover, our study has highlighted metabolic links in mitochondrial structure, function and redox status between mothers and their offspring during gestation that are altered by endurance training of the mothers. To our knowledge, this is the first clear demonstration that training of the mother has an impact on the bioenergetic functioning of the offspring. How the effects of training are transmitted is still unclear but the involvement of some epigenetic mechanism should be considered as it has been shown that ROS in the mother can induce changes in the fetus (Hitchler and Domann, 2007). Mitochondria could then support gene expression alterations through chromatin modifications that could persist in the next generation. These considerations require further longitudinal studies.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: H.D., C.B., K.C.; Methodology: F.S., H.D., I.H., C.B., K.C.; Software: F.S., I.H., C.B., K.C.; Validation: H.D., C.B., K.C.; Formal analysis: F.S., H.D., I.H., C.Q., G.V., A.G., E.F., C.B., K.C.; Investigation: F.S., H.D., I.H., C.Q., G.V.,

A.G., C.B., K.C.; Resources: C.B., K.C.; Data curation: C.B., K.C.; Writing - original draft: H.D., C.B., K.C.; Writing - review & editing: H.D., L.C., E.F., C.B., K.C.; Visualization: C.B., K.C.; Supervision: C.B., K.C.; Project administration: C.B., K.C.; Funding acquisition: C.B., K.C.

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