Physical activity prevents alterations in mitochondrial ultrastructure and glucometabolic parameters in a high-sugar diet model

Karina Barbosa de Queiroz 1, Kinulpe Honorato-Sampaio 2, Joamyr Victor Rossoni Júnior 3, Diego Andrade Leal 1, Angélica Barbosa G. Pinto 4, Lenice Kappes-Becker 4, Elisio Alberto Evangelista 1, Renata Guerra-Sá 1

1 Laboratório de Bioquímica e Biologia Molecular, Núcleo de Pesquisas em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil, 2 Faculdade de Medicina, Campus JK, Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brasil, 3 Laboratório de Bioquímica Metabólica, Núcleo de Pesquisas em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil, 4 Centro de Esportes, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil

* karinabq@gmail.com

Abstract

Endurance exercise is a remarkable intervention for the treatment of many diseases. Mitochondrial changes on skeletal muscle are likely important for many of the benefits provided by exercise. In this study, we aimed to evaluate the effects that a regular physical activity (swimming without workload) has on mitochondrial morphological alterations and glucometabolic parameters induced by a high-sugar diet (HSD). Weaned male Wistar rats fed with a standard diet or a HSD (68% carbohydrate) were subjected to 60 minutes of regular physical activity by swimming (without workload) for four- (20 sessions) or eight-week (40 sessions) periods. After training, animals were euthanized and the sera, adipose tissues, and skeletal muscles were collected for further analysis. The HSD increased body weight after an 8-week period; it also increased the fat pads and the adipose index, resulting in glucose intolerance and insulin resistance (IR). Transmission electron microscopy showed an increase in alterations of mitochondrial ultrastructure in the gastrocnemius muscle, as well as a decrease in superoxide dismutase (SOD) activity, and an increase in protein carbonylation. Regular physical activity partially reverted these alterations in rats fed a HSD, preventing mitochondrial morphological alterations and IR. Moreover, we observed a decrease in Pgc1α expression (qPCR analysis) in STD-EXE group and a less pronounced reduction in HSD-EXE group after an 8-week period. Thus, regular physical activity (swimming without workload) in rats fed a HSD can prevent mitochondrial dysfunction and IR, highlighting the crucial role for physical activity on metabolic homeostasis.
Introduction

Obesity prevalence in childhood has dramatically increased in the last three decades, as well as the morbidity and mortality risks later in life [1–4]. Although the disease is considered a multifactorial disorder and it is often associated with a high-fat diet intake, excessive consumption of sugar in early life has been described as a lipogenic modulator, affecting critical periods during childhood and promoting obesity in young adults [5–8]. Related to this nutritional behaviour, current evidence indicates that a decrease in children’s daily physical activity, has contributed to the increase in obesity prevalence around the world [1–3].

It is noteworthy that physical activity induces positive adaptations for metabolic homeostasis, which could lead to significant changes in lifestyle, and could also allow us to identify molecular responses that may be useful as both therapeutic targets and for exercise prescription [9]. Besides, training adaptation in skeletal muscle is deeply influenced by a number of factors such as regularity, intensity, and diet. For instance, a regular exercise program can increase mitochondrial density and function in muscle [10–15]. Mitochondrial biogenesis is strongly induced by endurance training [16–18] and occurs via coordinated gene expression, being the expression of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) the critical node for signalling to mitochondrial biology, inducing mitochondrial biogenesis and oxidative capacity [19–21]. These observations have led to the widespread statement that PGC1α is required for exercise-induced mitochondrial biogenesis [18,22–24]. On the other hand, a decrease in mitochondrial content (due to reduced mitochondrial biogenesis or damaged mitochondria accumulation) can result in mitochondrial dysfunction, which hinders the mitochondrial capability to function properly [25,26].

Mitochondrial dysfunction was first described in the context of glucose intolerance 40 years ago [27]. The majority of studies in this area since that time have focused on mitochondrial changes in skeletal muscle, once obese and diabetic patients have impaired mitochondrial function in this tissue [28]. It is noteworthy that reactive oxygen species (ROS) can lead to mitochondrial dysfunction [29], as well as mitochondria can be both the primary source of ROS and the primary target of ROS damage [30,31]. Moreover, pathological conditions such as obesity may increase ROS production [32,33]. Thereby, mitochondrial dysfunction induced by ROS could result in diminished fuel oxidation, mostly fatty acids, and a consequential accumulation of by-products of lipid metabolism, comprising diacylglycerols (DAG) and ceramides (CER) [25]. It may impair mitochondrial function, activating a vicious cycle that culminates in insulin resistance (IR) [26,34].

Previous studies performed by our group have demonstrated the effects of endurance training on a treadmill on adipose tissue of weaned rats fed with a high-sugar diet (HSD) in two different periods (4-week and 8-week periods) [35, 36]. It is worth mentioning that the achievement of exercise intensity and duration ensured that a significant endurance training adaptation had been produced. At first, we showed that rats fed a HSD and subjected to endurance training for an 8-week period had an impairment in the relationship between mRNA levels of uncoupling protein 1 and 3 (Ucp1/ Ucp3). This change may result in lower energy efficiency and may explain the increase in the adipose index observed in these animals [35]. Thereafter, we have investigated the molecular mechanism behind weight gain in trained rats fed a HSD, and have purposed a pleiotropic effect of leptin on white adipose tissue, via its receptor OB-Rb. The down regulation observed in OB-Rb after an 8-week period of endurance exercise avoided lipolysis in retroperitoneal white adipose tissue (rWAT) and promoted triacylglycerol (TAG) synthesis and storage. As a result, we observed high levels of serum leptin and an increase in the total mass of rWAT [36].

Because of these considerations, it is noteworthy that physical activity has been of great importance on the metabolic syndrome treatment, since exercise training improves glucose...
tolerance and reduces IR. Furthermore, exercise training at early ages can be more effective in metabolic control due to the energetic expenditure, which impairs body weight gain and, consequently, leads to a reduced lipid storage and an improvement in oxidative balance [37, 38]. However, the adaptation to exercise depends on aspects such as training load, frequency, and duration [39]. It is remarkable that the treadmill endurance training in our previous works was not able to reduce the HSD-induced metabolic impairment [35,36], pointing to a crucial role of the training type to set exercise benefits in obese and diabetic patients, including children. Moreover, the effects of a regular physical activity program (without a workload) on rats fed a HSD have not been elucidated yet.

Considering that skeletal muscle is an important tissue in metabolic control and is very modified by exercise training, in this study we aimed to elucidate the mitochondrial and glucometabolic changes induced by regular physical activity (swimming without workload) in rats fed a HSD. We hypothesized that regular physical activity may induce mitochondrial biogenesis via Pgc1α expression, preventing alterations in mitochondrial ultrastructure, thus improving the IR induced by a HSD. According to our results, regular physical activity has a protective effect in obese animals fed a HSD, up regulating Pgc1α expression and preventing mitochondrial dysfunction and IR after an 8-week period.

Material and methods
Animals and diet
All of the experimental procedures were authorised by the Ethical Committee for Animal Care of the Federal University of Ouro Preto (Protocol 099/2013) and were conducted in accordance with the regulations described in the Committee’s Guiding Principles Manual.

Four-week-old weaned male Wistar rats (60–65 grams) were bred at Centro de Ciência Animal (CCA, Federal University of Ouro Preto, Ouro Preto, MG, Brazil), housed in individual cages under controlled light (5–19 hours) [35, 36] and temperature (24±2°C) conditions; water and rat chow were provided ad libitum. The animals in the experimental groups were fed a HSD (68% carbohydrates) consisting of 33% standard chow (Nuvilab CR1, Colombo, Brazil), 33% condensed milk, and 7% sucrose by weight (the remainder percentage consisted of water) for 4-week and 8-week periods [40]. The control groups were fed only standard chow (STD). The composition of each diet has been previously published [35].

Rats had their calorie intake and body weight measured once a week during the experimental period. After 4-week and 8-week periods, the weekly food intake was multiplied by the energy density for the STD (12.22 kJ/g) and the HSD (13.31 kJ/g), to calculate the energy intake.

Experimental design
Before the beginning of the experimental procedures, 96 male Wistar rats were randomly divided into the following four groups: 1) sedentary rats fed a standard chow (STD-SED, standard diet–sedentary; N = 24), 2) swimming rats fed a standard chow (STD-EXE, standard diet–exercise; N = 24), 3) sedentary rats fed a high-sugar diet (HSD-SED, high-sugar diet–sedentary; N = 24), and 4) swimming rats fed a high-sugar diet (HSD-EXE, high-sugar diet–exercise; N = 24). Thereafter, the four groups were divided into two different swimming periods: 4-week (N = 12) and 8-week periods (N = 12). Physical activity started at the same time as the introduction of the HSD. At the end of their respective swimming periods, the animals in each group were euthanized by decapitation and the sera, adipose tissues (retroperitoneal, epididymal, and inguinal), and skeletal muscles (gastrocnemius and soleus) were collected. The gastrocnemius muscle was chosen to assess mitochondrial and oxidative damage due to its fibre-type, richness in glycolytic fibres, and responsiveness to diet-effects [41], while the soleus was
used to measure the oxidative capacity induced by regular physical activity [39]. To evaluate the development of obesity, the adiposity index was calculated with the following equation: 

\[ \text{100} \times \left( \frac{\text{sum of fat pad weights (g)}}{\text{body weight (g)}} \right) \] 

[42]. To calculate the fat pad weight, we used all the white adipose tissues that were removed and they were represented as relative weight: 

\[ \frac{\text{fat pad weight (g)}}{\text{body weight (g)}} \] 

\times 100. The physical activity protocol was carried out without a workload.

**Regular physical activity protocol**

The physical activity protocol was performed in a clear glass swimming pool (175 cm × 53 cm × 65 cm), in which they could see outside, the other rats swimming, and could orient themselves to their environment. Each rat swam in an individualized compartment, avoiding climbing behaviour, and in warm water (between 30 and 32°C), as previously published [39]. After each session of swimming, the rats were dried to prevent decreases in body temperature.

The regular exercise began with an adaptation period. The first training session lasted 2 minutes, and increased daily (10, 20, and 40 minutes) until reaching 60 minutes on the fifth day of the adaptation week. After this period, exercised groups carried out swim sessions of 60 minutes, five times per week, during 4-week (20 swimming sessions) or 8-week periods (40 swimming sessions). Non-continuous behaviour, like floating or diving, was avoided [43]. Sedentary groups underwent 2 minutes sessions during the experimental period. The animals were euthanized 24 hours after completion of the swimming protocol, in a fasted state.

**Sera parameters and Oral Glucose Tolerance Test (OGTT)**

Plasma glucose (N = 12) levels were measured using a Random Access Clinical Analyser (Wienner Lab, CM 200, São Paulo, Brazil) with Labtest kit (Labtest Diagnóstica SA, Minas Gerais, Brazil). Serum insulin concentration (N = 12) was assayed using a rat insulin ELISA (Millipore, São Paulo, Brazil).

The OGTT was performed in fasted rats (12 hours). Glucose levels from tail blood samples were monitored at 0, 30, 60, 90, and 120 min after gavage, using an Accu-Check glucometer (Accu-Chek Active, Roche Diagnostics Corp, Hague Road, IN), according to a previously published protocol [44]. The data were presented as the area under the curve (AUC). Finally, we calculated an insulin resistance index (HOMA) using the formula: (fasting serum insulin × plasma glucose)/ 22.5 [45].

**Citrate synthase assay**

The biomarker of oxidative metabolism in soleus muscle tissues was measured using a Citrate Synthase Assay kit (Sigma-Aldrich, St. Louis, MI), using a previously published protocol [35]. Briefly, tissue samples were homogenized (50 mM Tris–HCl, 1 mM EDTA, and 0.01 mM phenylmethylsulphonyl fluoride; pH 7.4) using a Polytron homogenizer, and then the homogenates were centrifuged (725 × g for 10 min at 4°C). The supernatant was decanted, and the citrate synthase activity was assayed according to the manufacturer’s protocol.

**Mitochondrial density**

For electron microscopy, posterior midbelly fragments of the gastrocnemius muscle, which are rich in white fibers [46], were dissected from three animals per group. The specimens were fixed in Karnovsky’s solution (2.5% glutaraldehyde and 2% paraformaldehyde) in 0.1 M cacodylate buffer pH 7.4 overnight at 4°C. They were post-fixed in a mixture of 2% (w/v) osmium tetroxide and 1.5% (w/v) potassium ferrocyanide for 2 hours to enhance contrast of organelles.
Specimens were washed in distilled water and kept in 2% uranyl acetate (en bloc staining) overnight. The samples were then serially dehydrated in graded ethanol baths and embedded in Epon 812. Specimens were sectioned in 50 nm ultrathin sections and stained with Reynolds lead citrate. Transmission electron microscopy (TEM) was performed using a FEI Tecnai G2-12 Spirit at 80 kV, which exhibits a point resolution of 0.49 nm. TEM was equipped with a SIS-MegaView 3 CCD camera and acquired images showed 1373 x 1070 pixels. Twenty-five electron micrographs per animal were taken at a ×11.000 magnification. Images were randomly selected from central parts of muscle fibers and were analysed with ImageJ [47]. Volume densities (Vv) of glycogen, lipid droplets, normal, and altered mitochondria were determined with the classic point counting method using a 130-point-grid (700 x 700 nm grid) projected onto each image (S1 Fig) [48, 49]. Altered mitochondria were defined as those that showed a swollen appearance with a rarefied matrix and damaged cristae, as previously described [16, 50].

**Superoxide Dismutase (SOD) activity and protein oxidation analysis**

The activity of the total antioxidant enzyme SOD was measured using a Superoxide Dismutase Assay Kit (cat# 706002; Cayman Chemical Company, Ann Arbor, MI). Briefly, 150 mg of gastrocnemius muscle samples were homogenized in cold 20 mM HEPES (pH 7.2) containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. Ten microliters of supernatant were used in the assay. The reaction was initiated by adding xanthine oxidase. The plate was incubated on a shaker for 20 min at 24˚C, and the absorbance at 450 nm was measured using a plate reader (ELX808 Absorbance Reader, Biotek, Winooski, VT). The SOD activity was expressed as U/mL per mg of tissue.

Protein oxidation by ROS leads to the formation of carbonyl derivatives, which can be measured by sensitive methods. Measurements of carbonylated protein were performed according to the method purposed by Levine [51]. Briefly, proteins were precipitated using trichloroacetic acid (TCA) (10%) and incubated in the dark with 2,4-dinitrophenylhydrazine (DNPH) and HCl at room temperature for 30 min. TCA (10%) was added to the precipitate and centrifuged at 4700 × g for 5 min at 4˚C. After discarding the supernatant, the precipitate was washed twice with ethanol/ethyl acetate (1:1 v/v) dissolved in a 6% sodium dodecyl sulphate (SDS) solution and centrifuged at 18000 × g for 10 min at 4˚C. Supernatant absorbance was measured at 370 nm. Carbonyl content was calculated using the DNPH molar extinction coefficient (21 × 10³ mol⁻¹.cm⁻¹), and the results were expressed as nanomoles of carbonyl groups per mg of protein. Total protein concentrations were determined using the BCA quantification method (Sigma-Aldrich, St. Louis, MI).

**Total RNA preparation and Pgc1α expression analysis by qRT-PCR**

Total RNA was obtained from the gastrocnemius muscle using a combination of Trizol reagent (Invitrogen, São Paulo, Brazil) and chloroform (Merck, São Paulo, Brazil) for extraction, according to the manufacturer’s protocol. Then, total RNA was purified on a column using the SV Total RNA Isolation System (Promega, São Paulo, Brazil), according to the manufacturer’s protocol. Total RNA was quantified using the NanoVue system (GE) and analysed by electrophoresis on a 1.2% agarose formamide-TBE (Tris/ Borate/ EDTA) gel. Total RNA was treated with RNase-free DNase I (Promega, São Paulo, Brazil) for 30 min, and the optical density of the solution was measured at 230, 260, and 280 nm. Ratios greater than 1.8 (260/280 and 260/230) were considered to be acceptable for gene expression quantification [52].

Two micrograms of total RNA were reverse transcribed into cDNA using the High Capacity cDNA RT kit (Applied Biosystems, São Paulo, Brazil). Then, mRNA expression was quantified by qRT-PCR using the SYBR Green system (Applied Biosystems, São Paulo, Brazil) and the
ABI 7300 Real-Time PCR System was used to detect the target. A rat-specific primer was used to detect \textit{Pgc1a} (NM_031347.1, \texttt{F-5'}-\texttt{GCACAACTCAGCAAGTCCTC-3'} and \texttt{R-5'}-\texttt{CCAAACAGCCGTAGACTG-3'}). Cycle thresholds were determined based on the SYBR Green emission intensity during the exponential phase. Cq data were normalized by \textit{rRNA 18S} (X01117.1 \texttt{F-5'}-\texttt{GTAAGTGCGGGTATA-3'} and \texttt{R-5'}-\texttt{CCATCCAATCGGTAGGC-3'}), which was stably expressed in all experimental groups. The relative gene expression was calculated using the $2^{-\Delta Cq}$ method [53].

Statistical analysis
Statistical analyses were performed using the Graph Pad Prism (version 6.01) software (Irvine, CA, USA). The sample size was determined with a power of 0.9 and a significance level ($\alpha$) of 0.05. The highest estimated size to assess our outcomes was chosen (N = 12). A Shapiro-Wilk test was used to verify data’s normalization. The data are reported as the mean ± standard deviation (S.D.). Differences between groups were evaluated using two-way ANOVA followed by Bonferroni test. $P$-values < 0.05 were considered statistically significant.

Results
Characteristic of animals submitted to regular physical activity and a HSD

Data related to the biometrical characteristic of experimental groups are shown in Tables 1 and 2. According to our results, calorie intake did not differ among groups, even in rats fed with a HSD (Tables 1 and 2). However, body weight was increased by diet ($P<0.05$) in a 4-week period, rising in the HSD-EXE group (Table 1). The interaction between diet and regular physical activity reduced the weight gain after an 8-week period ($P<0.001$), decreasing in the STD-EXE group (as compared to the sedentary control and when compared to HSD-EXE group) (Table 2).

Regarding adipose mass, the HSD increased epididymal, retroperitoneal, and inguinal pads from the 4-week period ($P<0.001$) (Table 1). In the same way, we observed an increase in

Table 1. Characteristics of rats fed a STD or HSD in a 4-week period.

|                      | 4-week period       |        |        |        | Effect of diet | Effect of exercise | Interaction |
|----------------------|---------------------|--------|--------|--------|----------------|-------------------|-------------|
|                      | STD-SED             | HSD-SED| STD-EXE| HSD-EXE|                |                   |             |
| Calorie intake (kJ)  | 329±76.9            | 303.5±76.9| 353.6±94.6| 330.8±90.2| 0.5496        | 0.5206           | 0.9732      |
| Body weight (g)      | 168.4±11.9          | 173.5±12| 154.4±20.5| 182.1±26.7*| 0.0195        | 0.6683           | 0.0987      |
| Epididymal (g)**     | 0.48±0.21           | 0.72±0.18| 0.5±0.12   | 0.68±0.14  | $P<0.001$     | 0.8325           | 0.5473      |
| Retroperitoneal (g)**| 0.2±0.08            | 0.5±0.07*| 0.3±0.1   | 0.4±0.2   | $P<0.001$     | 0.9607           | 0.5949      |
| Inguinal (g)**       | 1.0±0.22            | 1.5±0.3*| 0.96±0.3  | 1.3±0.44  | $P<0.001$     | 0.4384           | 0.6987      |
| Adipose index        | 1.7±0.4             | 2.6±0.4*| 1.6±0.5   | 2.4±0.7*  | $P<0.001$     | 0.3307           | 0.6713      |
| CS activity          | 5.6±0.8             | 5.6±0.8 | 16.5±3.6*| 8.76±2.4* | 0.0049        | $P<0.001$        | 0.0049      |

*Denotes statistically significant differences compared with the standard diet (STD) group (sedentary or trained) and exercise (trained or sedentary), followed by Bonferroni post hoc analyses.

**Relative weight: [fat pad weight (g)/ body weight (g)] × 100. CS: citrate synthase.

doi:10.1371/journal.pone.0172103.t001
epididymal ($P < 0.05$), retroperitoneal ($P < 0.001$) and inguinal ($P < 0.05$) depots induced by the HSD after an 8-week period, rising in HSD-SED and in HSD-EXE groups (as compared to their respective STD controls). The interaction between diet and regular physical activity decreased the retroperitoneal fat pad in the STD-EXE group (when compared to its untrained control) (Table 1). As a result, the adipose index was increased by diet ($P < 0.001$) in HSD groups (sedentary or trained), raising the amount of body fat from 4-week period.

Our results showed that citrate synthase (CS) activity was influenced by diet ($P < 0.05$), regular physical activity ($P < 0.001$), and the interaction ($P < 0.05$) in a 4-week period (Table 1), increasing its levels in STD-EXE group and decreasing in HSD-EXE groups (as compared to STD-EXE). After an 8-week period, the effect of the HSD had disappeared, and the CS activity increased by regular physical activity and the interaction between both variables ($P < 0.05$), rising in the STD-EXE group (Table 2).

**Glucometabolic parameters induced by regular physical activity and a HSD**

Fig 1 summarizes the metabolic parameters analysed in this study. Glucose levels were reduced by regular physical activity after an 8-week period ($P < 0.05$), although the post-hoc analysis did not show any statistically significant differences among groups (Fig 1A). In order to evaluate the metabolic response to diet sugar, we performed an OGTT, which was altered by the HSD ($P < 0.05$) from a 4-week period (Fig 1B). We observed an increase in the AUC in the HSD-SED group after 4-week and 8-week periods. Regardless of the fact that the HSD impaired oral glucose tolerance, the interaction between diet and regular physical activity decreased this parameter in the HSD-EXE group (as compared to the sedentary control) after an 8-week period ($P < 0.05$).

Insulin levels were increased by diet after an 8-week period ($P < 0.05$), rising in the HSD-SED group. Furthermore, regular physical activity and the interaction were able to block the HSD effect ($P < 0.05$), reducing the insulin levels of the HSD-EXE group (as compared to its sedentary control) (Fig 1C). The HOMA-IR was calculated to assess insulin resistance. Likewise to insulin levels, HOMA-IR was increased by diet after an 8-week period ($P < 0.05$), rising

---

Table 2. Characteristics of rats fed a STD or HSD in an 8-week period.

| 8-week period | STD-SED | HSD-SED | STD-EXE | HSD-EXE | Effect of diet | Effect of exercise | Interaction |
|---------------|---------|---------|---------|---------|---------------|-------------------|-------------|
| Calorie intake (kJ) | 361.5±44.9 | 327.8±87.8 | 413.7±66 | 313.9±58.8 | 0.1015 | 0.6135 | 0.3901 |
| Body weight (g) | 260.3±14.5 | 235.3±14.8 | 210.1±17 | 257±20.8* | 0.2045 | 0.1057 | $P < 0.001$ |
| Epididymal (g)** | 0.82±0.17 | 1.2±0.34* | 0.7±0.25 | 1.5±0.58* | 0.045 | 0.5866 | 0.3092 |
| Retroperitoneal (g)** | 0.7±0.13 | 1.2±0.28* | 0.4±0.19 | 1.4±0.13* | $P < 0.001$ | 0.7975 | 0.0460 |
| Inguinal (g)** | 1.4±0.27 | 2.4±0.84* | 1.2±0.29 | 2.9±0.67* | $P < 0.001$ | 0.5945 | 0.3091 |
| Adipose index | 2.9±0.5 | 5.2±1.45* | 2.4±0.8 | 5.8±1.2* | $P < 0.001$ | 0.6628 | 0.1790 |
| CS activity | 6.8±1.5 | 10.4±2.4 | 13.7±3.3 | 10.8±2.5 | 0.7755 | 0.0036 | 0.088 |

Data are expressed as means ± S.D. Statistically significant differences were determined using a two-way ANOVA to examine the effects of diet (HSD or STD) and exercise (trained or sedentary), followed by Bonferroni post hoc analyses.

*Denotes statistically significant differences compared with the standard diet (STD) group (sedentary or trained).

**Denotes statistically significant differences compared with its untrained control group (STD-SED or HSD-SED).

STD-SED, sedentary standard chow diet; HSD-SED, sedentary high-sugar diet; STD-EXE, exercised standard chow diet; HSD-EXE, exercised high-sugar diet.

**Relative weight: [fat pad weight (g)/ body weight (g)] × 100. CS: citrate synthase.

doi:10.1371/journal.pone.0172103.t002
in the HSD-SED group (as compared to STD-SED group). Regular physical activity and the interaction also blocked the HSD effect \((P<0.05)\), decreasing the HOMA-IR in the HSD-EXE group (as compared to its sedentary control) (Fig 1D).

Ultra-structural response in the gastrocnemius muscle promoted by regular physical activity and a HSD

Volumetric density of glycogen and lipids in gastrocnemius muscle fibres were measured to determine muscle fuel selection induced by regular physical activity using TEM (Fig 2A and 2B). Regarding glycogen, the volumetric density was increased by diet and regular physical activity \((P<0.001)\) (Fig 2A), demonstrating an increase in HSD-SED and STD-EXE groups (when compared to STD-SED group) in a 4-week period. The interaction decreased glycogen volumetric density in the HSD-EXE group (when compared to its untrained control, and when compared to STD-EXE group) after a 4-week period \((P<0.001)\). After the 8-week period, glycogen density was influenced by the same parameters as the 4-week period \((P<0.001)\). However, there was a decrease in the HSD-SED group as compared to the STD control.
Glycogen volumetric density

Lipid volumetric density

Fig 2. Effects of a high-sugar diet and regular physical activity (swimming without workload) on glycogen and lipids of gastrocnemius muscle fibres over 4-week and 8-week periods. (A) Quantification of glycogen and (B) lipids densities in gastrocnemius muscle fibres over 4-week (left panels) and 8-week (right panels) periods. N = 75 fields from three animals per group. Data are expressed as means ± S.D. Statistically significant differences were determined using a two-way ANOVA to examine the effects of diet (HSD or STD) and regular physical activity (trained or sedentary), followed by Bonferroni post hoc analyses; P<0.05 was considered statistically significant. *Denotes statistically significant differences compared to the standard diet (STD) group (sedentary or trained), # denotes statistically significant differences compared to its untrained control (STD-SED or HSD-SED). STD-SED, sedentary standard chow diet; HSD-SED, sedentary high-sugar diet; STD-EXE, exercised standard chow diet; HSD-EXE, exercised high-sugar diet.

doi:10.1371/journal.pone.0172103.g002

(P<0.001), whereas regular physical activity and the interaction increased glycogen density in the STD-EXE group and in the HSD-EXE group (as compared to its untrained control and when compared to STD-EXE group) (Fig 2A).

Lipid density was reduced by diet, decreasing in the HSD-SED group (P<0.001). Regular physical activity and the interaction increased lipid density in the HSD-EXE group (as compared to its untrained control, and when compared to the STD-EXE group) in a 4-week period (P<0.001) (Fig 2B). After the 8-week period, the lipid density was influenced by the same parameters as the 4-week period (P<0.001). Diet increased the lipid density in the HSD-SED group, regular physical activity increased the lipid density in the STD-EXE group, whereas the interaction increased the lipid density in the HSD-EXE group (as compared STD-EXE group) (Fig 2B).

Total mitochondria density in the gastrocnemius muscle is shown in Fig 3. In a 4-week period, we observed the effect of the diet (P<0.001) and the interaction, which increased the density of total mitochondria in the HSD-EXE group, as compared to HSD-SED group and when compared to STD-EXE group (Fig 3A). After the 8-week period, the diet increased the total mitochondria density in the HSD-SED group (as compared to STD-SED) (P<0.05), whereas the regular physical activity and the interaction increased the total mitochondria density in the STD-EXE group, when compared to its untrained control (P<0.001) (Fig 3C).

The altered mitochondria density is shown in Fig 4. These mitochondria showed a rarefied matrix and/or swollen appearance, and a disarrangement of cristae that became peripherally positioned [16, 50]. We observed an increase in the altered mitochondria density induced by the HSD (P<0.05) after an 8-week period (as compared to STD-SED group).
Measurements of oxidative stress induced by regular physical activity and a HSD

The oxidative stress in our model was indirectly measured on two levels: accessing the antioxidant enzyme activity (SOD) and the formation of carbonyl derivatives due to protein oxidation by ROS (Fig 5) [54].

SOD activity was decreased by diet ($P<0.001$), reducing its levels in HSD-SED and HSD-EXE groups in a 4-week period. After the 8-week period, the enzyme activity was reduced by diet ($P<0.001$), decreasing in both HSD-SED and HSD-EXE groups; however, the interaction between diet and regular physical activity increased the SOD activity in the HSD-EXE group (as compared to HSD-SED group) ($P<0.001$) (Fig 5A).

Protein carbonylation was increased by diet ($P<0.001$) in HSD-SED and HSD-EXE groups in the 4-week period. After the 8-week period, the carbonyl groups per mg of protein were
increased by diet \( (P<0.001) \), rising in both HSD-SED and HSD-EXE groups; however, regular physical activity and the interaction decreased the carbonylated proteins in the HSD-EXE group (as compared to HSD-SED group) \( (P<0.01) \) (Fig 5B).

**Effect of regular physical activity and a HSD on Pgc1α expression**

Fig 6 shows mRNA levels of Pgc1α in the gastrocnemius muscle. According to our results, regular physical activity had a significant effect \( (P<0.01) \) in Pgc1α expression during the 4-week
period, but there were no post hoc differences (Fig 6A). After the 8-week period, both diet ($P<0.01$) and interaction ($P<0.001$) had a significant effect in $Pgc1\alpha$ expression, inducing a down regulation of $\sim 2.6$-fold in the STD-EXE group (as compared to its sedentary control); however, we also observed an $\sim 1.8$-fold increase in $Pgc1\alpha$ expression in the HSD-EXE group when as compared to STD-EXE group (Fig 6B).

Fig 5. Effects of a high-sugar diet and regular physical activity (swimming without workload) on skeletal muscle oxidative stress over 4-week and 8-week periods. (A) Superoxide dismutase activity assay in gastrocnemius muscle over 4-week (left panels) and 8-week (right panels) periods. (B) Carbonyl groups per mg of protein in gastrocnemius muscle over 4-week (left panels) and 8-week (right panels) periods. $N = 6$. Data are expressed as means ± S.D. Statistically significant differences were determined using a two-way ANOVA to examine the effects of diet (HSD or STD) and regular physical activity (trained or sedentary), followed by Bonferroni post hoc analyses; $P<0.05$ was considered statistically significant. *Denotes statistically significant differences compared with the standard diet (STD) group (sedentary or trained), #denotes statistically significant differences compared with its untrained control (STD-SED or HSD-SED). STD-SED, sedentary standard chow diet; HSD-SED, sedentary high-sugar diet; STD-EXE, exercised standard chow diet; HSD-EXE, exercised high-sugar diet.

doi:10.1371/journal.pone.0172103.g005

Fig 6. Effects of a high-sugar diet and regular physical activity (swimming without workload) on $Pgc1\alpha$ expression over 4-week and 8-week periods. (A) $Pgc1\alpha$ levels in the gastrocnemius muscle. Gene expression profiles of the groups were evaluated using the $2^{-\Delta\Delta Ct}$ method. rRNA $18S$ was used as a reference gene. Data are expressed as means ± S.D. Statistically significant differences were determined using a two-way ANOVA to examine the effects of diet (HSD or STD) and regular physical activity (trained or sedentary), followed by Bonferroni post hoc analyses; $P<0.05$ was considered statistically significant. *Denotes statistically significant differences compared with the standard diet (STD) group (sedentary or trained), #denotes statistically significant differences compared with its untrained control (STD-SED or HSD-SED).

doi:10.1371/journal.pone.0172103.g006
Discussion

A regular exercise program has a number of health benefits, including enhancement of cardiovascular function, muscle metabolism, mitochondrial function, and work capacity [55], in addition to preventing risk factors associated with chronic disease, thus improving overall health and lifespan [56]. However, previous data from our group have demonstrated that the association between a treadmill endurance exercise (with a workload) and a HSD during an 8-week period was not able to reduce the HSD-induced metabolic impairment [35,36], suggesting that the exercise type plays a crucial role the benefits it confers. In the present study, our main finding was that rats undergoing a regular physical activity (swimming without workload) for 8-week period resulted in an improvement on the HSD-induced IR, preventing mitochondrial morphology alterations, as well as reverting the SOD activity decreases and the carbonylated proteins increases observed in HSD sedentary group. Our results suggested a protective effect of regular physical activity in obese animals fed a HSD, preventing mitochondrial dysfunction and IR, recovering the reduction observed in Pgc1α induced by regular physical activity, up regulating the gene expression after an 8-week period (when compared to STD-EXE group). However, regular physical activity down regulates Pgc1α levels, suggesting that the mechanism behind the mitochondrial biogenesis observed in our model is not related to the PGC1α expression induced by swimming (without workload), in contrast to the prevalent assumption in the field.

First, although the caloric intake did not differ among groups, a HSD results in body weight increases after an 8-week period, regardless of physical activity, increasing the fat pads and the adipose index. In general, this is an expected effect of the HSD. In other words, when glucose availability increases, an insulin-induced inhibition of lipolysis occurs, resulting in decreased fatty acid oxidation and free plasma fatty acids availability [57], thus increasing fatty acids synthesis and storage. Previous results from our group have demonstrated that endurance training on a treadmill (a moderate-intensity exercise, with a workload) was not able to reduce the HSD-induced increase in body fat [35,36]. One plausible explanation was the lower energy efficiency in trained rats fed a HSD, which resulted in body fat accumulation due to an impairment in the relationship between Ucp1/ Ucp3 [35]. Also, previously studies have shown that the low-intensity exercise (like ours, swimming without workload) was not effective in preventing neither the abdominal adiposity nor the increase in plasma and liver triglycerides associated with high-sucrose intake [58]. In addition, it was described that the relationship between exercise intensity and a reduction in visceral adipose tissue is “dose-dependent”, which means that there is a threshold for exercise intensity that determines the improvement in body composition [59]. However, regular physical activity reduced weight gain and body fat in animals fed a standard diet after an 8-week period. This result suggests that fat is the predominant source of fuel during a regular physical activity; which leads to an overall increase of the lipolysis rate, releasing fatty acids that are directly oxidized by mitochondria [60].

The adaptation of skeletal muscle oxidative capacity induced by exercise is well established and is considered a worthy indicator for exercise training efficiency [39]. Therefore, we evaluated CS activity in the soleus muscle as a parameter of endurance conditioning, only to assess if regular physical activity was able to increase the oxidative metabolism in the skeletal muscle. Along this line, CS activity levels demonstrated that regular physical activity was able to increase the aerobic capacity of rats fed a control diet (STD), which is consistent with the effect of physical activity on the oxidative metabolism in the soleus muscle [39]. On the other hand, animals fed a HSD and undergoing regular physical activity showed a reduction in CS activity levels after 4 weeks, which was lost after a 8-week period, suggesting a short effect of the HSD upon overall fat oxidation in our exercise type (swimming without workload) [61]. It was reported
that HSD may be associated with skeletal muscle loss in older male rats [62]. Although our rats are younger, this assertive could suggest a reduction in skeletal muscle mass induced by HSD and the exercise intensity is not enough to compensate this damage at 4-week period, which reduces the CS activity in our model. After 8-week period this effect upon HSD-EXE group disappears, since skeletal muscle possibly became acclimated to a regular physical activity and the exercise might recover this loss in a small scale. In addition, the exercise type may control muscle fuel selection, increasing fatty acid oxidation while temporarily shutting down glucose oxidation, increasing muscle glucose uptake and replenishing muscle glycogen stores to prepare for the next bout of exercise [63]. This corroborates our findings, were we observe an increase in glycogen storage after an 8-week period of regular physical activity in rats fed either a STD or a HSD. We also observed an increase in the intramuscular lipid density; it has been related that the skeletal muscle from endurance-trained subjects show an increase in intramuscular lipid content, as well as an increase in the muscle oxidative capacity [64]. This phenomenon is known as the athlete’s paradox [65], since the excessive accumulation of intramuscular lipid is related to IR. However, a recent work have shown that the exercise in mice fed a high-fat diet may increase the lipid content in skeletal muscle and brown these depot, which increase the fat oxidation during exercise [66].

It is noteworthy that exercise strongly induces mitochondrial biogenesis [16–18], and PGC1α induces mitochondrial biogenesis in the skeletal muscle [19–21]; additionally, PGC1α expression in skeletal muscle is induced by exercise [67]. We observed an increase in mitochondrial density after an 8-week period of regular physical activity, which is consistent to the role of exercise inducing mitochondrial biogenesis. These results corroborate the idea that regular physical activity increases total mitochondria [16–18]. However, expression levels of Pgc1α were down regulated in our model during the same period, in contrast to the established statement in the field [67]. A research conducted by Perry et al. [67] demonstrated that a single bout of a high-intensity interval training (7 days) was sufficient to up regulate PGC1α expression in skeletal muscle, whereas an increase in the markers of mitochondrial biogenesis could only be observed after the third bout. Interestingly, Pgc1α levels returned to baseline between each bout and the induction of the gene was lessened with each bout [67]. Rowe et al. [68] demonstrated using voluntary running wheel endurance exercise and PGC1α muscle-specific knockout mice (Myo-PGC-1aKO) that Pgc1α is dispensable within skeletal muscle for exercise-induced mitochondrial adaptations. They also suggested that Pgc1α expression does not necessarily indicate a causal relationship with mitochondrial biogenesis, which corroborates our findings. Therefore, this is the first time that Pgc1α expression in gastrocnemius muscle is described in a model of regular physical activity (swimming without workload). Moreover, it is also the first time that its down regulation is correlated to an increase in total mitochondria, indicating that other pathways may exist [68]. Although further experiments might be necessary to elucidate the full mechanism related to mitochondrial biogenesis in a model of regular physical activity, it is noteworthy that the p38c mitogen-activated protein kinase (MAPK) pathway (but not p38a or b) [69] and the AMP-activated protein kinase (AMPK) have been identified as important pathways in exercise-induced mitochondrial biogenesis [70].

It is worth mentioning that the current study only evaluates mitochondrial adaptations as a result of regular physical activity, and we cannot rule out that these adaptations could result from other types of exercise, such as endurance or strength training, which might be mediated by PGC1α expression. We have chosen this type of physical activity (low-intensity swimming) due to our previously data showed that endurance training (moderate intensity exercise) does not improve metabolic parameters in rats fed a HSD [35,36]. Moreover, we used a 4-week period to determine whether this period was sufficient to detect the beginning of the changes induced by regular physical activity for the parameters assessed.
According to our results, a HSD intake during an 8-week period, results in glucose intolerance and IR, as well as in an increase in the volumetric density of mitochondria with rarefied matrix and unstructured cristae (altered mitochondria). This architectural disorganization is associated with mitochondrial dysfunction [25]. It is noteworthy that obesity may increase ROS production, which causes oxidative modifications that lead to mitochondrial dysfunction [32,33,71]. In addition, mitochondrial dysfunction has been involved in IR development, likely the result of ROS production and accumulation of by-products of lipid metabolism [14,15,72]. We evaluated ROS production indirectly by measuring the activity of the antioxidant enzyme, SOD, and the product of protein oxidation by ROS, the carbonylated protein [54]. In our study, the excessive supply of sugar increased total mitochondria, suggesting a “compensatory” increase in the mitochondrial oxidative capacity in response to dietary sugar oversupply, in a similar way that was described for rats fed a high-fat diet [73, 74]. However, our results suggest that the magnitude of these increases was not sufficient to cope with the enhanced lipid availability induced by the HSD because we observed an increase in mitochondrial morphological alterations; thus, there is still ectopic lipid accumulation and IR [73, 74]. Moreover, it is noteworthy that it is imperative for the skeletal muscle to discriminate and carefully remove damaged mitochondria [26]. This is a consequence of the interaction between PGC1α and transcription factors and nuclear receptors, including nuclear respiratory factor-1 (NRF-1) [75], NRF-2 [76], and myocyte enhancer factor 2 (MEF2) [77, 78], which are related to mitochondrial dynamics. Even though we did not conduct any experiments to evaluate mitochondrial fusion and fission processes, our results suggest that these processes are impaired by a HSD once there is an accumulation of altered mitochondria [26]. Although we did not find a significant correlation between SOD activity and carbonylated proteins (unpublished data), our results showed a decrease in SOD activity and an increase in the carbonylated protein in rats fed a HSD, suggesting a role for oxidative stress in the HSD-induced mitochondrial dysfunction and IR development. More direct evidence connecting IR and mitochondrial ROS generation originated from a study by Anderson et al. [29], where the authors demonstrated that, in both rodents and humans, a high-fat diet increased the H$_2$O$_2$-emitting potential of mitochondria without any changes in oxidative capacity, supporting the role for oxidative stress in the development of IR [29].

Surprisingly, regular physical activity prevented the occurrence of these changes in rats fed a HSD after an 8-week period, promoting an improvement in glucometabolic parameters and preventing this architectural disorganization. Additionally, regular physical activity partially reverted the decrease in SOD activity and the increase in carbonylated proteins (which was not observed at 4-week period), suggesting a reduction in ROS production (which was indirectly measured) when compared to the sedentary control in the same period. An increase in mitochondrial function has been associated with a reduction in diabetes and obesity outcomes, and exercise has a major impact on both mitochondrial function and insulin sensitivity in skeletal muscle [18]. We observed that regular physical activity had a main effect in Pgc1α levels in rats fed a HSD and we also observed an increase in total mitochondria after a 4-week period. This finding is consistent with the fact that exercise is a potent up-regulator of Pgc1α expression, which may result in mitochondrial biogenesis [19–21, 67]. A 12-week exercise intervention program significantly increased muscle mitochondrial respiration and content in type 2 diabetes patients [79]. Similarly, an 8-week cycling exercise regime increased muscle fatty acid oxidative capacity and, in parallel, improved insulin-mediated glucose disposal [80]. Although the exercise types used were different, these findings corroborate the idea of the regular physical activity influences mitochondrial function and insulin sensitivity.

After an 8-week period, Pgc1α levels in trained rats fed a HSD were higher than those in trained rats fed a standard diet. This finding suggests that regular physical activity in rats fed a HSD may promote alterations in mitochondrial dynamics, conferring positive impacts on
metabolic functions of skeletal muscle. In other words, although a HSD is metabolically harmful, regular physical activity can induce a functional improvement in the mitochondrial network, meaning that regular physical activity induces muscle cells to not only generate new mitochondria, but also maintain the healthy ones and remove the damaged ones [26]. Although we did not conduct any experiments to determine mitochondrial dynamics, our results suggest that regular physical activity in rats fed a HSD favours the muscle to recognize and selectively remove damaged mitochondria, thus maintaining the healthy ones, which are necessary to contain the IR in our model. Therefore, the efficient removal of altered mitochondria is critical in preserving mitochondrial function; in other words, mitochondrial biogenesis is important but it may not be the only regulatory event that leads to an improved function of the mitochondrial network [26] and insulin sensitivity in skeletal muscle in our model.

Thus, we have demonstrated that regular physical activity (swimming without workload) in rats fed a HSD can ameliorate the HSD-induced mitochondrial morphological alterations and glucometabolic parameters, suggesting a crucial role for regular physical activity on preserving mitochondrial function, inducing positive adaptations for metabolic homeostasis. In addition to significant changes in lifestyle, the exercise type can also suggest different molecular responses in a metabolic context, which can help us to use distinct exercise approaches to aim at different therapeutic targets.

**Supporting information**

S1 Fig. Representative structures analysed in the study by transmission electron microscopy. Arrows: Mitochondria irregularly shaped with sectioned tubular cristae in various planes. Arrowhead: Glycogen as electron dense granules (25–30 nm in diameter) found in inter and intramyofibrillar space as isolated particles or in clusters. LD: Lipid droplet showing homogeneous texture and no bounding membrane. Asterisks: myofibrils composed by thick filaments assembled from myosin II molecules and thin filaments from actin. (EPS)

**Acknowledgments**

The authors would like to acknowledge the Center of Microscopy at the Universidade Federal de Minas Gerais (http://www.microscopia.ufmg.br) for providing the equipment and technical support for experiments involving electron microscopy.

**Author Contributions**

- **Conceptualization:** KBQ.
- **Formal analysis:** KBQ.
- **Funding acquisition:** KHS RGS.
- **Investigation:** KBQ KHS JVRJ DAL ABGP EAE.
- **Methodology:** KBQ KHS JVRJ DAL ABGP LKB.
- **Project administration:** KBQ.
- **Resources:** LKB RGS.
- **Supervision:** RGS.
- **Visualization:** KBQ.
References

1. Stephen A, Alles M, de Graaf C, Fleith M, Hadjilucas E, Isaacs E, et al. The role and requirements of digestible dietary carbohydrates in infants and toddlers. Eur J Clin Nutr. 2012; 66(7):765–79. doi: 10.1038/ejcn.2012.27 PMID: 22473042

2. Guran T, Bereket A. International epidemic of childhood obesity and television viewing. Minerva Pediatr. 2011; 63(6):483–90. PMID: 22075803

3. Hackman CL, Knollden AP. Theory of reasoned action and theory of planned behavior-based dietary interventions in adolescents and young adults: a systematic review. Adolesc Health Med Ther. 2014; 5:101–14. doi: 10.2147/AHMT.S56207 PMID: 24966710

4. Bluhé S, Schwarz P. Metabolically healthy obesity from childhood to adulthood—Does weight status alone matter? Metabolism. 2014.

5. Joslowski G, Goletzké J, Cheng G, Gunther AL, Bao J, Brand-Miller JC, et al. Prospective associations of dietary insulin demand, glycemic index, and glycemic load during puberty with body composition in young adulthood. Int J Obes (Lond). 2012.

6. McDevitt RM, Bott SJ, Harding M, Coward WA, Bluck LJ, Prentice AM. De novo lipogenesis during controlled overfeeding with sucrose or glucose in lean and obese women. Am J Clin Nutr. 2001; 74(6):737–46. PMID: 11722954

7. de Queiroz KB, Coimbra RS, Ferreira AR, Carneiro CM, Paiva NC, Costa DC, et al. Molecular mechanism driving retroperitoneal adipocyte hypertrophy and hyperplasia in response to a high-sugar diet. Molecular nutrition & food research. 2014; 58(12):2331–41.

8. Skledar MT, Milosevic M. Breastfeeding and time of complementary food introduction as predictors of obesity in children. Central European journal of public health. 2015; 23(1):26–31. doi: 10.21101/cejph.a3956 PMID: 26036095

9. Barbieri E, Agostini D, Polidori E, Potenza L, Guescini M, Lucertini F, et al. The pleiotropic effect of physical exercise on mitochondrial dynamics in aging skeletal muscle. Oxid Med Cell Longev. 2015; 917085. doi: 10.1155/2015/917085 PMID: 25945152

10. Heilbronn LK, Gan SK, Turner N, Campbell LV, Chisholm DJ. Markers of mitochondrial biogenesis and metabolism are lower in overweight and obese insulin-resistant subjects. J Clin Endocrinol Metab. 2007; 92(4):1467–73. doi: 10.1210/jc.2006-2210 PMID: 17244782

11. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A. 2003; 100(14):8466–71. doi: 10.1073/pnas.1032913100 PMID: 12832613

12. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes. 2005; 54(1):8–14. PMID: 15616005

13. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes. 2002; 51(10):2944–50. PMID: 12351431

14. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. The American journal of physiology. 1999; 277(6 Pt 1): E1130–41.

15. Kim JY, Hickner RC, Corrigh RL, Dohm GL, Hournard JA. Lipid oxidation is reduced in obese human skeletal muscle. American journal of physiology Endocrinology and metabolism. 2000; 279(5):E1039–44. PMID: 11052958

16. Golnick PD, King DW. Effect of exercise and training on mitochondria of rat skeletal muscle. The American journal of physiology. 1969; 216(6):1502–9. PMID: 5786739

17. Freyssenet D, Berthon P, Denis C. Mitochondrial biogenesis in skeletal muscle in response to endurance exercises. Archives of physiology and biochemistry. 1996; 104(2):129–41. doi: 10.1076/apab.104.2.129.12878 PMID: 8818195

18. Little JP, Saldaar A, Benton CR, Wright DC. Skeletal muscle and beyond: the role of exercise as a mediator of systemic mitochondrial biogenesis. Applied physiology, nutrition, and metabolism = Physiologie appliquée, nutrition et métabolisme. 2011; 36(5):588–607. doi: 10.1139/h11-076 PMID: 21888528

19. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, et al. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature. 2002; 418(6899):797–801. doi: 10.1038/ nature00904 PMID: 12181572
20. Wende AR, Schaeffer PJ, Parker GJ, Zechner C, Han DH, Chen MM, et al. A role for the transcriptional coactivator PGC-1alpha in muscle refueling. The Journal of biological chemistry. 2007; 282(50):36642–51. doi: 10.1074/jbc.M700706200 PMID: 17932032

21. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, et al. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. The Journal of biological chemistry. 2003; 278(29):26597–603. doi: 10.1074/jbc.M301850200 PMID: 12734177

22. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, et al. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2002; 16(14):1879–86.

23. Baar K. Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and 2, and PPAR alpha in the adaptive response to endurance exercise. The Proceedings of the Nutrition Society. 2004; 63(2):269–73. doi: 10.1079/PNS2004334 PMID: 15294042

24. Ljubicic V, Joseph AM, Saleem A, Ugucconi G, Collu-Marchese M, Lai RY, et al. Transcriptional and post-transcriptional regulation of mitochondrial biogenesis in skeletal muscle: effects of exercise and aging. Biochimica et biophysica acta. 2010; 1800(3):38–45. PMID: 19682549

25. Montgomery MK, Turner N. Mitochondrial dysfunction and insulin resistance: an update. Endocr Connect. 2015; 4(1):R1–R15. doi: 10.1530/EC-14-0092 PMID: 25385852

26. Yan Z, Lira VA, Greene NP. Exercise training-induced regulation of mitochondrial quality. Exercise and sport sciences reviews. 2012; 40(3):159–64. doi: 10.1097/JES.0b013e3182575999 PMID: 22732425

27. Yamada T, Ida T, Yamaoka Y, Ozawa K, Takasan H, Honjo I. Two distinct patterns of glucose intolerance in icteric rats and rabbits. Relationship to impaired liver mitochondria function. The Journal of laboratory and clinical medicine. 1975; 86(1):38–45. PMID: 1151141

28. Stump CS, Henriksen EJ, Wei Y, Sowers JR. The metabolic syndrome: role of skeletal muscle metabolism. Annals of medicine. 2006; 38(6):389–402. doi: 10.1080/0785389060088413 PMID: 17008303

29. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, et al. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. The Journal of clinical investigation. 2009; 119(3):573–81. doi: 10.1172/JCI37048 PMID: 19186863

30. Harman D. The biologic clock: the mitochondria? J Am Geriatr Soc. 1972; 20(4):145–7. PMID: 5016631

31. Miquel J, Economos AC, Fleming J, Johnson JE. Mitochondrial role in cell aging. Exp Gerontol. 1980; 15(6):575–91. PMID: 7009178

32. Gomez-Cabrera MC, Domenech E, Viñ a J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med. 2008; 44(2):126–31. doi: 10.1016/j.freeradbiomed.2007.02.001 PMID: 18119748

33. Gomez-Cabrera MC, Martinez A, Santangelo G, Pallardó FV, Sastre J, Viñ a J. Oxidative stress in marathon runners: interest of antioxidant supplementation. Br J Nutr. 2006; 96 Suppl 1:S31–3.

34. Morino K, Petersen KD, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes. 2006; 55 Suppl 2:S9–S15.

35. de Queiroz KB, Rodovalho GV, Guimaraes JB, de Lima DC, Coimbra CC, Evangelista EA, et al. Endurance training blocks uncoupling protein 1 up-regulation in brown adipose tissue while increasing uncoupling protein 3 in the muscle tissue of rats fed with a high-sugar diet. Nutrition research. 2012; 32(9):709–17. doi: 10.1016/j.nutres.2012.06.020 PMID: 23084644

36. de Queiroz KB, Guimaraes JB, Coimbra CC, Rodovalho GV, Carneiro CM, Evangelista EA, et al. Endurance training increases leptin expression in the retroperitoneal adipose tissue of rats fed with a high-sugar diet. Lipids. 2014; 49(1):85–96. doi: 10.1007/s11745-013-3854-7 PMID: 24243000

37. Pitts GC. Body composition in the rat: interactions of exercise, age, sex, and diet. The American journal of physiology. 1984; 246(2 Pt 2):R495–501.

38. Radak Z, Kaneko T, Tahara S, Nakamoto H, Ohno H, Sasvari M, et al. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. Free radical biology & medicine. 1999; 27(1–2):69–74.

39. Evangelista FS, Brum PC, Krieger JE. Duration-controlled swimming exercise training induces cardiac hypertrophy in mice. Brazilian journal of medical and biological research. – Revista brasileira de pesquisa s medicas e biologicas / Sociedade Brasileira de Biofisica [et al]. 2003; 36(12):1751–9.

40. de Lima DC, Silveira SA, Haibara AS, Coimbra CC. The enhanced hyperglycemic response to hemorrhage hypotension in obese rats is related to an impaired baroreflex. Metabol Brain Dis. 2008; 23(4):361–73. doi: 10.1007/s11011-008-9101-x PMID: 18836821

41. Bhatt BA, Dube JJ, Dedousis N, Reider JA, O’Doherty RM. Diet-induced obesity and acute hyperlipidemia reduce IkappaBalpha levels in rat skeletal muscle in a fiber-type dependent manner. American
42. Taylor BA, Phillips SJ. Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics. 1996; 34(3):389–98. doi: 10.1006/geno.1996.0302 PMID: 8786140

43. Kregel KC, Allen, D. L., Booth, F. W., Fleshner, M. R., Henriksen, E. J., et al. Exercise protocols using rats and mice. Resource book for the design of animal exercise protocols: American Physiological Society; 2006. p. 35–40.

44. Santos SH, Fernandes LR, Mario EG, Ferreira AV, Porto LC, Alvarez-Leite JI, et al. Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. Diabetes. 2008; 57(2):340–7. doi: 10.2337/db07-0953 PMID: 18025412

45. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412–9. PMID: 3899825

46. Ustunel I, Demir R. A histochemical, morphometric and ultrastructural study of gastrocnemius and soleus muscle fiber type composition in male and female rats. Acta anatomica. 1997; 158(4):279–86. PMID: 9416359

47. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature methods. 2012; 9(7):671–5. PMID: 22930834

48. Weibel ER, Staubli W, Gnagi HR, Hess FA. Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereologic methods, and normal morphometric data for rat liver. The Journal of cell biology. 1969; 42(1):68–91. PMID: 4891915

49. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. CMAJ. 2006; 174(6):801–9. doi: 10.1503/cmaj.051351 PMID: 16534088

50. Sidossis LS, Stuart CA, Shulman GI, Lopaschuk GD, Wolfe RR. Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. The Journal of clinical investigation. 1996; 98(10):2244–50. doi: 10.1172/JCI119034 PMID: 8941640

51. Mitendorfer B, Klein S. Physiological factors that regulate the use of endogenous fat and carbohydrate fuels during endurance exercise. Nutrition research reviews. 2003, 16(1):97–108. doi: 10.1079/NRR200357 PMID: 19079940

52. Chu L, Riddell MC, Takken T, Timmons BW. Carbohydrate intake reduces fat oxidation during exercise in obese boys. Eur J Appl Physiol. 2011; 111(12):3135–41. doi: 10.1007/s00421-011-1940-1 PMID: 21468747

53. Gatinneau E, Savary-Auzelouix I, Migne C, Polakof S, Dardevet D, Mosni L. Chronic Intake of Sucrose Accelerates Sarcopenia in Older Male Rats through Alterations in Insulin Sensitivity and Muscle Protein Synthesis. The Journal of nutrition. 2015; 145(5):923–30. doi: 10.3945/jn.114.198420 PMID: 25896812

54. de Souza MO, Silva M, Silva ME, Oliveira Rde P, Pedrosa ML. Diet supplementation with acai (Euterpe oleacea Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats. Nutrition. 2010; 26(7–8):804–10. doi: 10.1016/j.nut.2009.09.007 PMID: 20022468
63. Finck BN, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest. 2006; 116(3):615–22. doi: 10.1172/JCI27794 PMID: 16511594

64. Horowitz JF, Klein S. Lipid metabolism during endurance exercise. The American journal of clinical nutrition. 2000; 72(2 Suppl):588–635. PMID: 10919960

65. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. The Journal of clinical endocrinology and metabolism. 2001; 86(12):5755–61. doi: 10.1210/jcem.86.12.8075 PMID: 11739435

66. Morton TL, Galior K, McGrath C, Wu X, Uzer G, Uzer GB, et al. Exercise Increases and Browns Muscle Lipid in High-Fat Diet-Fed Mice. Frontiers in endocrinology. 2016; 7:80. doi: 10.3389/fendo.2016.00080 PMID: 27445983

67. Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. The Journal of physiology. 2010; 588(Pt 23):4795–810. doi: 10.1113/jphysiol.2010.199448 PMID: 20921196

68. Rowe GC, El-Khoury R, Patten IS, Rustin P, Arany Z. PGC-1alpha is dispensable for exercise-induced mitochondrial biogenesis in skeletal muscle. PloS one. 2012; 7(7):e41817. doi: 10.1371/journal.pone.0041817 PMID: 22846187

69. Pogozelski AR, Geng T, Li P, Yin X, Lira VA, Zhang M, et al. p38gamma mitogen-activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. PloS one. 2009; 4(11):e7934. doi: 10.1371/journal.pone.0007934 PMID: 19936205

70. O’Neill HM, Maarbjergh SJ, Crane JD, Jeppesen J, Jorgensen SB, Schertzer JD, et al. AMP-activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. PloS one. 2009; 4(11):e7934. doi: 10.1371/journal.pone.0007934 PMID: 19936205

71. HARMAN D. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 1956; 11:298–300. PMID: 13332224

72. Simouneau JA, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 1999; 13(14):2051–60.

73. Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, et al. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(22):7815–20. doi: 10.1073/pnas.0802057105 PMID: 18509063

74. Turner N, Kowalski GM, Leslie SJ, Raisis S, Yang C, Lee-Young RS, et al. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. Diabetologia. 2013; 56(7):1638–48. doi: 10.1007/s00125-013-2913-1 PMID: 23620060

75. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmann G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell. 1999; 98(1):115–24. doi: 10.1016/S0092-8674(00)80611-X PMID: 10412986

76. Mootha VK, Handschin C, Arlow D, Xie X, St Pierre J, Sihag S, et al. ERRalpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(17):6570–5. doi: 10.1073/pnas.0401401101 PMID: 15100410

77. Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelmann G, Lehmann JJ, et al. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98(7):3820–5. doi: 10.1073/pnas.061035098 PMID: 11274399

78. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(12):7111–6. doi: 10.1073/pnas.123352100 PMID: 12764228

79. Phielix E, Meex R, Moonen-Kompis E, Hesselink MK, Schrauwen P. Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals. Diabetologia. 2010; 53(8):1714–21. doi: 10.1007/s00125-010-1764-2 PMID: 20422397

80. Rimbert V, Boirie Y, Bedu M, Hocquette JF, Ritz P, Morio B. Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2004; 18(6):737–9.