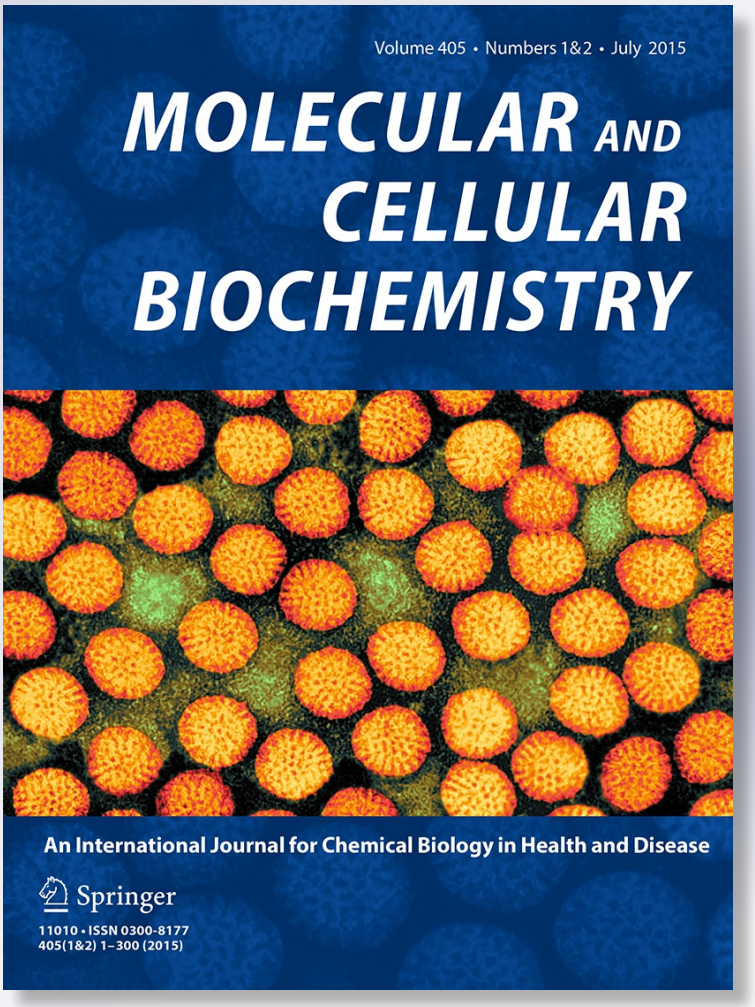
Transcriptional modulation of mitochondria biogenesis pathway at and above critical speed in mice

L. Mille-Hamard, C. Breuneval, A. S. Rousseau, P. Grimaldi & V. L. Billat

Molecular and Cellular Biochemistry
An International Journal for Chemical Biology in Health and Disease

ISSN 0300-8177
Volume 405
Combined 1-2

Mol Cell Biochem (2015) 405:223-232
DOI 10.1007/s11010-015-2413-3



Springer

Mol Cell Biochem (2015) 405:223–232
DOI 10.1007/s11010-015-2413-3

Transcriptional modulation of mitochondria biogenesis pathway at and above critical speed in mice

L. Mille-Hamard[1] · C. Breuneval[1] · A. S. Rousseau[2] · P. Grimaldi[2] · V. L. Billat[1]

Received: 28 December 2014 / Accepted: 18 April 2015 / Published online: 26 April 2015

Abstract High- or moderate-intensity endurance training leads to mitochondrial biogenesis via the peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α)/mitochondrial transcription factor A (Tfam) signaling pathway. Although this pathway is stimulated during acute exercise, the relationship between its activity and the intensity of the exercise has not been characterized. In animal studies, individualized running speeds have not previously been assessed. Here, we sought to determine whether this pathway was modulated after a bout of exhaustive exercise at different relative intensities (at and over critical speed (CS)). Our starting hypotheses were that (i) exercise-induced overexpression of PGC-1α in skeletal muscle falls at intensities above CS, and (ii) transcriptional activity of the mitochondrial biogenesis signaling cascade is intensity-sensitive at and above CS. To test these hypothesis, male Friend Virus B-Type mice were divided into a control group and three exercise groups (exercising at CS, peak velocity (vPeak) and 150 % CS, respectively). mRNA expression levels for genes involved in mitochondrial biogenesis signaling were analyzed in the quadriceps muscle. PGC-1α was overexpressed at all exercise intensities. We also identified that, PGC-1α mRNA expression was negatively correlated with exercise intensity and blood lactate levels but not with maximal oxygen uptake, vPeak, or CS. Expression of the PGC-1α co-activator peroxisome proliferator-activated receptor β was negatively correlated with the exercise intensity. In contrast, expression levels of Tfam were dissociated from exercise intensity. Our data indicate that at the intensities used in endurance training, the expression of mitochondrial biogenesis genes is finely modulated by the relative intensity of exhaustive exercise.

Keywords Exercise · Muscle · Mitochondria biogenesis

Abbreviations

$\dot{V}O_{2max}$	Maximal oxygen uptake
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator-1α
CS	Critical speed
PPARβ	Peroxisome proliferator-activated receptor beta
Tfam	Mitochondrial transcription factor A
Sirt-1	Sirtuin 1
VO_2	Oxygen consumption
vPeak	Peak velocity
[La] rest	Blood lactate concentration at rest
C_t	Critical threshold
AMPK	AMP-activated protein kinase
miRNA	microRNA

Introduction

Endurance training is known to induce an increase in maximal oxygen uptake ($\dot{V}O_{2max}$), which is an overall measure of the pulmonary, cardiovascular, and muscle

✉ L. Mille-Hamard
laurence.hamard@inserm.fr; laurence.hamard@univ-evry.fr

[1] UBIAE, INSERM U902, Université Evry-Val d'Essonne, Bd Francois Mitterrand, Batiment Maupertuis, 91025 Evry, France

[2] C3M, INSERM 1065, Université Nice-Sophia-Antipolis, Nice, France

systems' joint ability to extract, transport, and use oxygen. This increase in $\dot{V}O_{2max}$ reflects (amongst other things) a rise in the number and activity of mitochondria [1, 2]. Endurance training is now based on a mixture of low-intensity, high volume exercise at and below critical speed (CS) with high-intensity intermittent exercises (above CS and even above maximal aerobic speed) and/or strength exercises. These combinations supposedly optimize aerobic performance (i.e., $\dot{V}O_{2max}$) [3, 4]. Given that increases in mitochondrial parameters (citrate synthase activity and respiration) coincide with a whole-body increase in $\dot{V}O_{2max}$ [2, 5, 6], one can suppose that a combination of low-intensity and high-intensity exercise increases mitochondria biogenesis. Furthermore, low-intensity, high-volume exercise helps to maintain mitochondrial content and function throughout life; whereas, inactivity is emerging as a major determinant of age-associated changes in the mitochondria [7]. It is now known that even a single, acute bout of exercise impacts the expression of a plethora of genes. However, it is not completely understood how mitochondrial biogenesis is regulated across a range of exercise intensities and thus how training can maximize this adaptive process. In the field of health and fitness, recent research has shown that resistance training can reduce obesity by promoting a negative energy balance and changing body fat distribution [8, 9]. Thus, it is physiologically relevant to seek to characterize the causal relationships between exercise characteristics, induced metabolic stress, and the molecular responses that lead to mitochondrial biogenesis under physiological and physiopathological conditions.

Although we are starting to understand molecular mechanisms that underlie contraction-induced mitochondrial biogenesis, the precise nature of the exercise stimulus that induces maximal mitochondria adaptation remains to be determined [10]. Nevertheless, it is clear that the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is the main transcriptional mediator of mitochondrial biogenesis and oxidative capacity in skeletal muscle [11, 12]. Furthermore, PGC-1α orchestrates the transcriptional activity of several nuclear transcription factors that are essential for mitochondrial biogenesis [13]. Levels of PGC-1α increase in response to acute exercise in humans and rodents [14–17]. However, it is not clear whether the magnitude of this transcriptional response is related to the magnitude of the stimulus (i.e., exercise intensity and/or duration). Furthermore, it appears that exercise intensity (i.e., light vs. moderate) increases the mRNA abundance in human skeletal muscle after an acute exercise bout [18], whereas high-intensity exercise in humans (such as short sprints) was associated with a modest increase in mRNA levels of PGC-1α [15, 19]. This suggests that overexpression is lower at supramaximal intensities. Interestingly, in animal model, Tadaishi et al. reported an intensity-dependent increase in PGC-1α expression in mice [20]. However, all the animal studies performed to date have involved exercise at the same absolute velocity. In contrast, Nordsborg et al.'s studies in humans [21] showed that relative workload has to be considered when studying exercise-induced mRNA transcription. Accordingly, we decided to study relative exercise intensity in rodents. In rodents, it has been shown that CS is an appropriate index of aerobic capacity [22, 23]. We therefore used CS as a measure of submaximal relative exercise intensity. The relationships between the relative exercise intensity, the level of metabolic stress, and the amplitude of PGC-1α mRNA expression at more than moderate exercise intensities have not been characterized. Although PGC-1α expression in response to exercise has been extensively studied, few researchers have investigated modulation of the signaling cascade upstream of mitochondrial biogenesis by exercise intensity or duration. Thus, it has not been established whether there is a relationship between exercise intensity and the magnitude of the increase in mRNA levels for components of the pathways leading to mitochondrial biogenesis, such as peroxisome proliferator-activated receptor beta (PPARβ), mitochondrial transcription factor A (Tfam), and sirtuin 1 (Sirt-1).

Thus, the objective of the present study was to characterize modulations of mRNA expression of PGC-1α and elements of the mitochondrial biogenesis signaling cascade after a single bout of exhaustive exercise, as a function of the latter's relative intensity. Thus, based on previous findings in the literature, we tested the hypotheses whereby (i) exercise-induced overexpression of PGC-1α within skeletal muscle falls at intensities above CS, and (ii) transcriptional activity of the mitochondrial biogenesis signaling cascade is intensity-sensitive at and above CS.

Materials and methods

Ethical approval

All the animal procedures described below were approved by the Institutional Animal Care and Use Committee at CERFE (Genopole, Evry, France) and complied with the Council of Europe's European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

All mice were purchased from Charles River Laboratories (Lyon, France), ear–punched for identification and housed in an accredited animal facility (CERFE, Genopole, Evry, France), with a specific and opportunist pathogen-

free environment, a temperature of 22 °C and 12:12 h light–dark cycles. Water and food were supplied ad libitum. A total of 44 adult male Friend Virus B-Type/N mice were included in the study. To avoid a potential gender effect, female mice were excluded. The mice were 5 months old when they started the exercise tests.

Exercise protocols

Devices

The exercise test protocol was performed on a single-lane motorized treadmill (Columbus Instruments, Columbus, OH) with an adjustable belt speed (0–99.9 m min^{-1}). The rear of the treadmill was equipped with a low-voltage, electric stimulating bar, to encourage each mouse to run. The bar was set to deliver 0.2 mA at a frequency of 0.25 Hz, which caused an uncomfortable shock but did not injure the animal.

Measurements and data recording

Oxygen consumption (VO_2) was measured by means of a rapid-flow, open-circuit, indirect calorimeter. The single-lane treadmill was placed in a metabolic chamber (Oxymax, Columbus Instruments) from which gas samples were withdrawn for analysis every 5 s and dried prior to measurement of the oxygen and carbon dioxide fractions.

The gas analyzers were calibrated with standardized gas mixtures (Air Liquide Santé, Paris, France) before every test session, as recommended by the manufacturer. The treadmill test provided an estimate of $\dot{V}O_{2max}$, defined as the highest VO_2 attained over a 15-s period during the test protocol.

Familiarization

The exercising mice were familiarized with the treadmill over a 1-week period via the completion of four 10-min running sessions at speeds ranging from 0 to 9 m min^{-1} (0, 3, 6 and 9 m min^{-1}). All mice succeeded in running for the required time at an intensity of 9 m min^{-1}. The velocity was not increased above this value, in order to avoid a training effect. The mice subsequently performed an incremental exercise test.

The incremental test load: peak velocity (vPeak), blood lactate levels, and $\dot{V}O_{2max}$ *determination*

Starting at a speed of 10 m min^{-1}, the exercise intensity was increased by 3 m min^{-1} every 3 min, with an incline of 0 %. Exercise continued until exhaustion, which was defined as the inability to maintain running speed despite contact with the electric grid for more than 5 s. The last stage completed by the mouse was defined as vPeak. Blood lactate levels were measured before exercise and 2 min after the end of the incremental exercise. After local antisepsis with alcohol, the distal 2 mm of the animal's tail was cut off. To avoid contamination, the first drop of blood was discarded. Next, a 5 μL blood sample was collected on a small test strip and the blood lactate concentration was measured with a Lactate Pro assay (Arkray Inc., Kyoto, Japan).

Steady-state exercise: determination of the CS

The protocol consisted of four constant-speed runs (18–51 m min^{-1}) leading to exhaustion within 1 h, as described previously [22]. A single trial was performed per day, and the protocol covered a period of 4 days. The time to exhaustion was recorded at each speed. Two parameters were used to estimate endurance performance: the distance (in meters) the mice were able to cover at a given speed and the time needed to cover the distance (in minutes). For each mouse, the CS was calculated from the slope (a) of the regression line of distance (y) against the time to exhaustion (x) in the four tests, according to the equation $y = ax + b$.

Acute exhaustive exercise

At least 2 weeks after the determination of CS, vPeak and $\dot{V}O_{2max}$, all animals (except those in the control group; $n = 10$) ran at their specific relative velocity until exhaustion in a continuous mode at either CS ($n = 8$) or vPeak ($n = 16$), or in an intermittent mode (150 % CS; $n = 10$). The 150 % CS group performed four bouts of exhaustive runs separated by 5-min rest periods, to ensure a sufficient duration. We recorded the time to exhaustion and the blood lactate concentrations before exercise and 2 min after the end of exercise at a steady-state intensity. Mice were sacrificed by cervical dislocation 2 h after their last run. The quadriceps muscles were quickly removed, immediately frozen in liquid nitrogen, and stored at −80 °C prior to molecular analysis.

Real-time quantitative PCR (RT-qPCR)

Total RNA was isolated from each muscle sample using a Qiagen RNA isolation kit (Qiagen, Hilden, Germany). The total RNA concentration and purity were measured spectrophotometrically according to the optical density at 260 nm and the 260/280 nm ratio, respectively (Nanodrop®, Thermo Scientific, Wilmington, DE, USA). RNA was considered to be pure when the 260/280 nm

absorbance ratio was close to 2. cDNA was prepared from RNA using Superscript II and oligo dT primers (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). RT-qPCR (using a 7300 Real-Time PCR System, Applied Biosystems) were performed in triplicate. The reaction volume contained 12 µL of SYBR® Green PCR Master Mix (Qiagen, Hilden, Germany), forward and reverse primers (Table 1), and the cDNA template. The primers were designed using Primer express software (Applied Biosystems). All RT-qPCR reactions were run with internal positive and negative controls. The reference gene was 36B4. The C_t values for 36B4 were the same for all experiments. The data were analyzed using the comparative critical threshold (C_t) method, where the amount of target gene (normalized against the amount of endogenous control gene) relative to the control group value is given by $2^{-\Delta\Delta C_t}$. This quantity is noted as a fold-change (relative to the control group) in the figures. We checked dissociation curves to ensure that a single PCR product had been amplified.

Statistics

The threshold for statistical significance was set to $p < 0.05$. A one-way analysis of variance was used to establish whether or not the performance phenotype differed from one group to another and to test the effect of exercise intensity on gene expression. When appropriate, a Bonferroni post hoc test was used to determine which mean values differed significantly from the others. A Spearman rank order test was used to identify correlations between variables.

Results

The mice's characteristics and performance phenotypes are reported in Table 2, and did not differ significantly from one subgroup to another. The mean absolute velocity of the acute run differed significantly when comparing the subgroups (25.1 ± 4 m min^{-1} for CS, 30.6 ± 5 for vPeak, and 39.7 ± 4 for 150 % CS; $p < 0.001$). The data characterizing the acute exercise bout in each running group are summarized in Table 3. The distance run, the time limit, and velocity varied significantly from one group to another ($p < 0.01$). The blood lactate concentration was significantly elevated after exercise (relative to rest) at all exercise intensities ($p < 0.05$). Furthermore, the blood lactate concentrations were significantly higher in the 150 % CS group than in the CS and vPeak groups ($p < 0.05$).

Table 2 Characteristics and performance of the mice

Characteristics ($n = 34$)	
Age (weeks)	21.3 ± 3.5
Weight (g)	33.7 ± 3.1
$\dot{V}O_{2max}$ (ml kg$^{-0.75}$ min^{-1})	51.5 ± 7.5
vPeak (m min^{-1})	30.6 ± 6.0
[La]rest (mmoL)	2.6 ± 0.7

Data are expressed as the mean ± SD

$\dot{V}O_{2max}$ maximal oxygen uptake, *vPeak* peak velocity, *[La]rest* blood lactate concentration at rest

A single bout of exhaustive exercise was associated with significantly higher levels of PGC-1α mRNA when performed at CS (a 8.05 ± 5.6-fold increase; $p = 0.02$), vPeak (a 6.32 ± 2.7-fold increase; $p < 0.001$), and 150 % CS (a 2.46-fold increase; $p < 0.001$), relative to non-exercising (control) mice (Fig. 1).

The magnitudes of the increases in PGC-1α mRNA levels at CS and vPeak did not differ, although there was a negative, linear correlation ($R^2 = 0.985$, $p < 0.05$) between the fold change in PGC-1-α mRNA levels on one hand and relative speed (Fig. 2a) on the other. Conversely, there was a positive correlation between the distance run and PGC-1α gene expression ($R^2 = 0.978$, $p < 0.05$). Moreover, the maximum blood lactate concentrations were negatively correlated with both PGC-1α expression ($R^2 = 0.897$, $p < 0.05$. Figure 2b) and the distance run ($R^2 = 0.991$, $p < 0.05$, Fig. 2c).

Considering the signaling pathway, we showed that Tfam was significantly downregulated 2 h after running at vPeak ($p = 0.01$) and 150 % CS ($p = 0.04$). In contrast, PPARβ and Sirt1 expression did not differ from control

Table 1 Primer sequences used for RT-qPCR

Gene	Forward primer	Reverse primer
36B4	TCCAGGTTTGGGCATCA	CTTTATCAGCTGCACATCACTCAGA
PGC-1α	GGACAGTCTCCCCGTCCAT	TCCATCTGTCAGTGCATCAAATG
Tfam	GCACCCTGCAGAGTGTTCAA	CGCCCAGGCCTCTACCTT
PPARβ	AGATGGTGGCAGAGCTATGACC	TCCTCCTGTGGCTGTTCC
Sirt-1	CCGCGGATAGGTCCATATACTT	TCGAGGATCGGTGCCAAT

Table 3 Variables characterizing the exercise sessions in each group

	CS	vPeak	150 % CS
Time limit (min)	76.9 ± 29.5[b,c]	36.7 ± 29.1[a,c]	7.3 ± 3.8[a,b]
Distance run (m)	1820.7 ± 566.2[b,c]	1007.8 ± 701[a,c]	281.6 ± 143.1[a,b]
Peak blood lactate (mmoL)	6.2 ± 2.6[c]	8.4 ± 4.0[c]	13.3 ± 1.4[a,b]
Speed (m min^{-1})	25.1 ± 4.1[b,c]	30.6 ± 5.0[a,c]	39.7 ± 3.7[a,b]

Values are expressed as the mean ± SD

[a] Differs significantly from CS

[b] Differs significantly from vPeak

[c] Differs significantly from 150 % CS

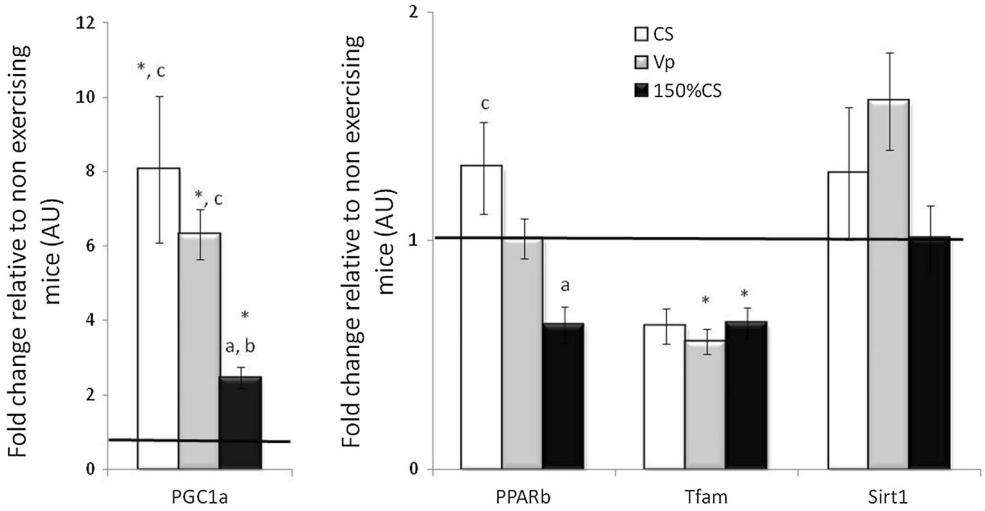


Fig. 1 Gene expression in response to a single bout of acute, exhaustive exercise. Gene expression of mitochondrial biogenic transcription factors and co-activators PGC-1α, PPARβ, Tfam, and Sirt-1 in response to a single, exhaustive bout of exercise (2 h post-exercise) at CS ($n = 6$–8), vPeak ($n = 14$–16) and 150 % CS ($n = 8$–10). The PGC-1α mRNA level increased after all exercise sessions, albeit to a lesser extent after the 150 % CS session. The PPARβ mRNA expression was significantly greater at 150 % CS than at CS. Tfam expression was lower at vPeak and 150 % CS. No differences were seen for Sirt-1 mRNA. Data are presented as the fold-change relative to non-exercising mice. Error bars represent the SEM. *Asterisk* differs significantly from control; **a** differs significantly from CS; **b** differs significantly from vPeak; **c** differs significantly from 150 % CS. $p < 0.05$

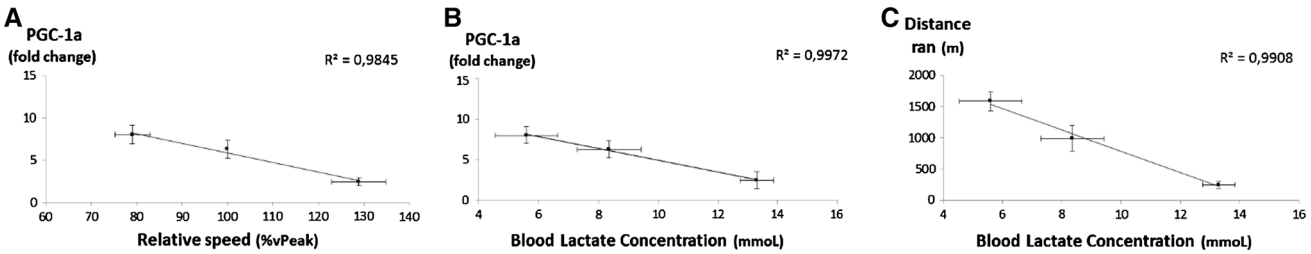


Fig. 2 Relationships between absolute running speed, PGC-1α mRNA levels, and the blood lactate concentration ($n = 26$). The PGC-1α mRNA level was negatively correlated with absolute running speed (**a**) and the blood lactate concentration (**b**). The distance run was negatively correlated with the blood lactate concentration (**c**). Data are represented as the mean ± SEM

values (Fig. 1). Furthermore, mRNA levels of PPARβ were significantly greater in the CS group than in the 150 % CS group (Fig. 1) and was negatively correlated with relative speed and the distance run ($p < 0.05$). Moreover, PPARβ expression was correlated with the expression of PGC-1α ($r = 0.67$, $p < 0.05$) and Tfam ($r = 0.41$, $p < 0.05$). However, there were no correlations between mRNA expression in the quadriceps on one hand and the mice's performance (vPeak, CS or $\dot{V}O_{2max}$) on the other.

Discussion

In the present study in mice, we assessed changes in the mRNA expression of PGC-1α and other genes involved in the mitochondrial biogenesis pathway after a single, acute bout of exercise performed at three different relative intensities at and above CS. Our major finding was that exhaustive exercise (ranging in intensity from CS to 150 % CS) induced PGC-1α overexpression; the latter was negatively correlated with relative exercise intensity and the blood lactate level but not with the animal's performance (i.e., neither vPeak, CS, nor $\dot{V}O_{2max}$). There was also a negative correlation between exercise intensity and mRNA expression levels of PPARβ (a PGC-1α co activator). Furthermore, expression of Tfam (which coordinates nuclear and mitochondria gene expression) decreased 2 h after the end of the exercise, regardless of the exercise intensity. Our data thus indicate that at the speeds used in endurance training (i.e., between the CS and the supramaximal intensity), the expression of genes involved in mitochondrial biogenesis is modulated by the relative intensity of the exhaustive exercise.

Exercise is known to increase PGC-1α mRNA levels in humans and rodents. However, few studies have looked at the putative relationship between exercise intensity and PGC-1α expression. In humans, PGC-1α mRNA levels rise with increasing intensity (from 40 % of the $\dot{V}O_{2max}$, i.e., below CS, to 80 % of $\dot{V}O_{2max}$, i.e., around CS), and a bell-shaped curve is observed between 73 and 133 % of $\dot{V}O_{2max}$ [24]. However, contradictory data have also been reported. Indeed, it seems that PGC-1α is more strongly expressed at high intensity (e.g., during a 30 s, all-out sprint) [25, 26] than at moderate intensity [10, 27]. However, firm conclusions cannot be drawn because the studies were heterogeneous in terms of the populations and exercise protocols (e.g., the time interval between the end of exercise and the tissue sampling). Using absolute speed, Tadaishi et al.'s study in mice found that PGC-1α mRNA levels were positively correlated with the intensity of treadmill running (10–30 m min^{-1}) [20]. However, PGC-1α expression above 30 m min^{-1} was not studied. By considering relative intensities from CS (25.1 ± 4.1 m min^{-1}) to 150 % CS (39.7 ± 3.7 m min^{-1}), we evidenced a negative correlation between relative speed and the transcription of the PGC-1α gene. The higher the exercise intensity, the lower the PGC-1α expression is—even though expression levels were always higher than in non-exercising controls. This is the first rodent study to have used relative intensities and high velocities (i.e., above CS). Our results indicate that in mice running at above CS, the overexpression of PGC-1α mRNA decreases as exercise intensity increases.

The duration of exercise may influence PGC-1α expression. Indeed, we found a correlation between the distance run and PGC-1α expression levels: the greater the distance run, the higher the expression is. This result is consistent with previous results in humans, for which no inter-trial differences were found for any of the genes studied after a fixed distance had been run at different intensities [10]. Furthermore, metabolic adaptations depend on the duration of exercise [28]. Furthermore, the lack of an increase in PGC-1α mRNA levels in Tobina et al.'s study [29] may have been due to a relatively short exercise protocol (90 min at around 50 % of VO_2peak), whereas exhaustive, lengthy (2–3 h) exercise of comparable intensity was associated with a strong increase in PGC-1α mRNA levels in a similar population [30, 31]. Consequently, exercise duration may have influenced mRNA expression in our study, since the distance and duration increased as the intensity decreased. Unfortunately, we did not observe a correlation between the duration of the run and the PGC-1 α expression level in any of the groups. It will be necessary to address this hypothesis in an experimental design that distinguishes between duration and intensity.

Several putative kinases (such as calcium/calmodulin-dependent protein kinase, mitogen-activated protein kinase p38, and AMP-activated protein kinase (AMPK)) regulate PGC-1α and are activated in an intensity-dependent manner [12, 15]. This activation may contribute to the peak in mRNA expression observed at CS. Above the lactate threshold, levels of phosphorylated AMPK increase with the exercise intensity. Furthermore, the lactate and epinephrine that are produced at these intensities are thought to increase PGC-1α expression [20, 29, 32]. Taken as a whole, these data imply that PGC-1α expression should be positively correlated with intensity—even above CS. Our observation of a negative correlation between PGC-1α mRNA and intensity above CS therefore suggests that other mechanisms may inhibit exercise-induced mRNA production at high intensities. This mechanisms may be related (at least in part) to the types of muscle fiber recruited at high intensities [24]. In exercise in the rat, the PGC-1α gene is upregulated in glycolytic muscles and downregulated in oxidative muscles [33]. However, researchers have not found a relationship between PGC-1α mRNA expression and muscle fiber type in humans [34, 35]. This aspect has not been studied in mice; it is therefore possible that fiber-type recruitment contributed to the correlation observed in the present study. Furthermore, long-lasting exercise is known to induce glycogen depletion. Thus, exercise at CS (for 76.9 ± 29.5 min, corresponding to 1820 ± 566 m) could have induced greater glycogen depletion in the CS group than in the other running groups (for which times and distances were significantly shorter). It has been reported that AMPK phosphorylation is elevated when

glycogen stores are depleted [36]. Thus, the exhaustiveness of the exercise performed by the mice probably accentuated glycogen depletion at CS and may explain both the positive correlation between the distance run and PGC-1α expression and the negative correlation found above CS. Lastly, microRNAs (miRNAs, i.e., small-noncoding RNAs) regulate gene expression by degrading mRNA or inhibiting the latter's translation [37, 38]. Some miRNAs seem to be involved in the translational regulation of PGC-1α, skeletal muscle metabolism, and mitochondrial biogenesis [39, 40]. It is not known whether acute exercise impacts these or other miRNAs or whether exercise intensity modulates miRNA levels. Thus, our present data suggest that several (probably overlapping and redundant) signaling pathways mediate the transcription of PGC-1α [13].

The present study's secondary objective was to determine whether exercise-induced expression of genes that are known to be implicated in mitochondrial biogenesis (along with PGC-1α) is related to exercise intensity. Mitochondrial biogenesis is a complex, multifactorial process that involves proteins encoded by nuclear DNA and proteins encoded by mitochondrial DNA [41]. It also requires the activation of Tfam and PPARs [42, 43]. Sirt-1 is a putative mitochondrial biomarker and was therefore monitored in the present study [44].

Tfam is essential for mitochondrial biogenesis, since it controls the transcription and replication of the mitochondrial genome [13, 45]. In our present study, we observed an intriguing decrease in Tfam mRNA levels after exercise (contrasting with the increase in PGC-1α mRNA levels) under all experimental conditions. There is no consensus in the literature on the relationship between exercise and expression of Tfam mRNA. In humans, some researchers have failed to observe a post-exercise effect on Tfam mRNA expression [34, 41, 46], whereas others observed an elevation [25, 47, 48]. However, a study in humans found that Tfam mRNA levels were depressed at the end of an exercise session [41]. In mice, a transient increase in Tfam expression was noted in the hours following a long-lasting session of endurance exercise [49]. Furthermore, Daussin et al. reported that a 5-day training period was long enough to induce an increase in Tfam expression in the rat [50]. Although our results contradict these data, this discrepancy might be explained by the fact that our mice ran at higher speeds (i.e., 25.1 ± 4.1 m min^{-1} for CS). Again, most other studies in rats and mice have featured moderate-intensity, endurance-like exercise, and little is known about acute adaptations to intense (supramaximal) exercise bouts. We are unable to say whether the observed decrease in Tfam mRNA levels was transient (due to the time interval between the end of the exercise and biopsy) or persisted over the longer term after acute exercise. Indeed, Popov et al. observed an increase in Tfam mRNA content at least 3 h after exercise in humans [51]. Tfam is trans-activated by nuclear respiratory factor 1 (Nrf-1). Following a single bout of exercise, Nrf-1 DNA binding increased after 12–18 h of recovery [47, 52]. Thus, our choice of the assay time point in the present study probably prevented us from seeing an increase in Tfam some hours after exercise. One can thus hypothesize that intensity-dependent changes in Tfam mRNA levels would have been detected if they had been investigated later in recovery. Consequently, the levels of Tfam and PGC-1α mRNA expression were not correlated 2 h after exercise. This dissociation has also been reported under other experimental conditions [51, 53]. Lastly, our data in mice suggest that there was at least a transient, exercise-induced decrease in Tfam mRNA expression, especially during high-intensity exercise (above vPeak). However, this decrease in Tfam expression was not correlated with expression levels of PGC-1α mRNA. The absence of an increase in Tfam mRNA and protein levels over time would indicate that the initiation of mitochondrial DNA transcription is blunted above CS-despite the increase in the PGC-1α response. This may explain why training intensity is not an important determinant of the number of mitochondria [54]. However, more evidence is needed before firm conclusion can be drawn. Modulations of mRNA stability, protein levels, and protein trafficking will have to be studied as a function of the exercise stimulus.

Animal studies and studies of human cell lines have shown that PPARβ is the most abundant PPAR isoform in skeletal muscle and acts as a PGC-1α cofactor [13, 26]. In mice, a short period of muscle unloading (mimicking disuse, such as bed rest in humans) or starvation is associated with increased PPARβ mRNA content [55, 56]. However, there is no evidence to suggest that acute exercise influences mRNA levels of PPARβ at the end of exercise in rodents, whatever the muscle type [33]. This is consistent with our finding that exercise did not modify PPARβ expression (relative to a non-exercising control group). In humans, it was shown that PPARβ mRNA levels increased for the first 4 h after the end of endurance exercise [14, 26, 31, 34, 57] and, to a lesser extent, at higher intensity. The lower increase at high intensity might also have been due to differences in the subjects' fitness levels [25]. Conversely, Wang et al. did not observe an increase in PPARβ expression after an hour of endurance exercise [35], suggesting that longer periods of exercise are needed for an increase in PPARβ mRNA levels. Indeed, we found a correlation between exercise duration and PPARβ expression ($p < 0.05$), as well as between PPARβ and PGC-1α expression. These results are consistent with lipid use during long-lasting exercise (for 76.9 ± 29.5 min at CS) and increased PPARβ expression. Indeed, all the ligands that activate PPARs are long-chain fatty acids or their

derivatives [58]. Although we observed an interesting correlation between PPARβ expression and the relative exercise speed (indicating an intensity-dependent response), the physiological meaning is unclear because the PPARβ expression did not differ from control experiments. Further studies are needed to better characterize the modulation of PPARβ levels (and notably the time course of mRNA expression) and protein translation at a range of exercise intensities at and above CS.

Lastly, we quantified Sirt-1-a known PGC-1α activator and a putative mitochondrial biomarker [44]. Sirt-1 is a NAD + sensing protein that activates PGC-1α by deacetylation [59]. Nuclear Sirt-1 activity is associated with contraction-stimulated mitochondrial biogenesis, and exercise increases Sirt-1 activity. Furthermore, Sirt-1 has been detected in the mitochondria, in close proximity to mitochondrial DNA [60–63]. It is not known whether the mRNA expression of Sirt1 increases with exercise and whether Sirt1 can modulates PGC-1α. In the present study, we did not observe an effect of exercise (and thus of exercise intensity) on Sirt1 transcript levels.

Our results show that the elements of the mitochondrial biogenesis signaling pathway are differentially regulated according to relative intensity of a single, acute, exhaustive bout of exercise. Further studies are needed to understand the time course of this response. As in humans, relative intensities should be applied in animal models. In addition to PGC-1α's role in the signaling pathways that induce mitochondrial biogenesis, this cofactor is also known to be involved in the downregulation of inflammation [42, 64]. Hence, relatively long durations of exercise (i.e., endurance exercise) should be prescribed in chronic inflammation-associated pathologies such as obesity, Alzheimer's disease, and cancer. However, more data are needed in order to determine the exercise intensity and duration that can optimize PGC-1α expression at the mRNA and protein levels. A low intensity has already been recommended for minimizing the harmful effects of exercise in sedentary subjects [65]. However, this hypothesis must be confirmed by recording long-term metabolic adaptations. Indeed, protein content, activity, and distribution across the various cellular compartments influence skeletal muscle adaptations [45, 47, 49, 66]. Future research must focus on (i) the early time course of molecular events in response to exercise and (ii) optimization of the exercise prescription as a function of the sporting or patient population.

Conclusion

In mice, expression of PGC-1α (the master regulator of the mitochondrial biogenesis pathway) and PPARβ was intensity-dependent above CS. In contrast, the transcription of Tfam and Sirt-1 was not modulated by the exercise intensity. Our results suggests that the complex response of the mitochondrial biogenesis pathway in muscles is finely modulated by exercise intensity and that the molecular signals differ across the range of exercise intensities used in endurance training (i.e., at and above CS). The exercise intensity that maximizes mitochondrial biogenesis remains to be determined.

Conflict of interest All authors have no conflicts of interest, in accordance with journal policy.

References

1. Nader GA (2006) Concurrent strength and endurance training: from molecules to man. Med Sci Sports Exerc 38(11):1965–1970. doi:10.1249/01.mss.0000233795.39282.33
2. Jacobs RA, Fluck D, Bonne TC, Burgi S, Christensen PM, Toigo M, Lundby C (2013) Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. J Appl Physiol 115(6):785–793. doi:10.1152/japplphysiol.00445.2013
3. Ziemann E, Grzywacz T, Luszczyk M, Laskowski R, Olek RA, Gibson AL (2011) Aerobic and anaerobic changes with high-intensity interval training in active college-aged men. J Strength Cond Res 25(4):1104–1112. doi:10.1519/JSC.0b013e3181d09ec9
4. Astorino TA, Allen RP, Roberson DW, Jurancich M (2012) Effect of high-intensity interval training on cardiovascular function, VO_2max, and muscular force. J Strength Cond Res 26(1):138–145. doi:10.1519/JSC.0b013e318218dd77
5. Tonkonogi M, Sahlin K (1997) Rate of oxidative phosphorylation in isolated mitochondria from human skeletal muscle: effect of training status. Acta Physiol Scand 161(3):345–353. doi:10.1046/j.1365-201X.1997.00222.x
6. Burgomaster KA, Hughes SC, Heigenhauser GJ, Bradwell SN, Gibala MJ (2005) Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. J Appl Physiol 98(6):1985–1990. doi:10.1152/japplphysiol.01095.2004
7. Lanza IR, Nair KS (2010) Mitochondrial metabolic function assessed in vivo and in vitro. Curr Opin Clin Nutr Metab Care 13(5):511–517. doi:10.1097/MCO.0b013e32833cc93d
8. Strasser B (2013) Physical activity in obesity and metabolic syndrome. Ann N Y Acad Sci 1281:141–159. doi:10.1111/j.1749-6632.2012.06785.x
9. Landry BW, Driscoll SW (2012) Physical activity in children and adolescents. PM & R 4(11):826–832. doi:10.1016/j.pmrj.2012.09.585
10. Bartlett JD, Joo CH, Jeong TS, Louhelainen J, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP (2012) Matched work high-intensity interval and continuous running induce similar increases in PGC-1alpha mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. J Appl Physiol 112(7):1135–1143. doi:10.1152/japplphysiol.01040.2011
11. Holloszy JO (2008) Regulation by exercise of skeletal muscle content of mitochondria and GLUT4. J Physiol Pharmacol 59(Suppl 7):5–18
12. Laursen PB (2010) Training for intense exercise performance: high-intensity or high-volume training? Scand J Med Sci Sports 20(Suppl 2):1–10. doi:10.1111/j.1600-0838.2010.01184.x

13. Uguccioni G, D'Souza D, Hood DA (2010) Regulation of PPARgamma coactivator-1alpha function and expression in muscle: effect of exercise. PPAR Res. doi:10.1155/2010/937123
14. Russell AP, Feilchenfeldt J, Schreiber S, Praz M, Crettenand A, Gobelet C, Meier CA, Bell DR, Kralli A, Giacobino JP, Deriaz O (2003) Endurance training in humans leads to fiber type-specific increases in levels of peroxisome proliferator-activated receptor-gamma coactivator-1 and peroxisome proliferator-activated receptor-alpha in skeletal muscle. Diabetes 52(12):2874–2881
15. Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M (2009) Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. J Appl Physiol 106(3):929–934. doi:10.1152/japplphysiol.90880.2008
16. Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O, Dohm GL, Yan Z, Newgard CB, Muoio DM (2005) Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. J Biol Chem 280(39):33588–33598. doi:10.1074/jbc.M507621200
17. Adser H, Wojtaszewski JF, Jakobsen AH, Kiilerich K, Hidalgo J, Pilegaard H (2011) Interleukin-6 modifies mRNA expression in mouse skeletal muscle. Acta Physiol 202(2):165–173. doi:10.1111/j.1748-1716.2011.02269.x
18. Egan B, Carson BP, Garcia-Roves PM, Chibalin AV, Sarsfield FM, Barron N, McCaffrey N, Moyna NM, Zierath JR, O'Gorman DJ (2010) Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. J Physiol 588(10):1779–1790. doi:10.1113/jphysiol.2010.188011
19. Serpiello FR, McKenna MJ, Bishop DJ, Aughey RJ, Caldow MK, Cameron-Smith D, Stepto NK (2012) Repeated sprints alter signaling related to mitochondrial biogenesis in humans. Med Sci Sports Exerc 44(5):827–834. doi:10.1249/MSS.0b013e318240067e
20. Tadaishi M, Miura S, Kai Y, Kawasaki E, Koshinaka K, Kawanaka K, Nagata J, Oishi Y, Ezaki O (2011) Effect of exercise intensity and AICAR on isoform-specific expressions of murine skeletal muscle PGC-1alpha mRNA: a role of beta(2)-adrenergic receptor activation. Am J Physiol Endocrinol Metab 300(2):E341–E349. doi:10.1152/ajpendo.00400.2010
21. Nordsborg NB, Lundby C, Leick L, Pilegaard H (2010) Relative workload determines exercise-induced increases in PGC-1alpha mRNA. Med Sci Sports Exerc 42(8):1477–1484. doi:10.1249/MSS.0b013e3181d2d21c
22. Billat VL, Mouisel E, Roblot N, Melki J (2005) Inter-and intrastrain variation in mouse critical running speed. J Appl Physiol 98(4):1258–1263. doi:10.1152/japplphysiol.00991.2004
23. Copp SW, Hirai DM, Musch TI, Poole DC (2010) Critical speed in the rat: implications for hindlimb muscle blood flow distribution and fibre recruitment. J Physiol 588(Pt 24):5077–5087. doi:10.1113/jphysiol.2010.198382
24. Edgett BA, Foster WS, Hankinson PB, Simpson CA, Little JP, Graham RB, Gurd BJ (2013) Dissociation of increases in PGC-1alpha and its regulators from exercise intensity and muscle activation following acute exercise. PLoS One 8(8):e71623. doi:10.1371/journal.pone.0071623
25. Psilander N, Wang L, Westergren J, Tonkonogi M, Sahlin K (2010) Mitochondrial gene expression in elite cyclists: effects of high-intensity interval exercise. Eur J Appl Physiol 110(3):597–606. doi:10.1007/s00421-010-1544-1
26. Wang L, Psilander N, Tonkonogi M, Ding S, Sahlin K (2009) Similar expression of oxidative genes after interval and continuous exercise. Med Sci Sports Exerc 41(12):2136–2144. doi:10.1249/MSS.0b013e3181abc1ec
27. Mathai AS, Bonen A, Benton CR, Robinson DL, Graham TE (2008) Rapid exercise-induced changes in PGC-1alpha mRNA and protein in human skeletal muscle. J Appl Physiol 105(4):1098–1105. doi:10.1152/japplphysiol.00847.2007
28. Le Moyec L, Mille-Hamard L, Triba MN, Breuneval C, Petot H, Billat VL (2012) NMR metabolomics for assessment of exercise effects with mouse biofluids. Anal Bioanal Chem 404(2):593–602. doi:10.1007/s00216-012-6165-6
29. Tobina T, Yoshioka K, Hirata A, Mori S, Kiyonaga A, Tanaka H (2011) Peroxisomal proliferator-activated receptor gamma co-activator-1 alpha gene expression increases above the lactate threshold in human skeletal muscle. J Sports Med Phys Fit 51(4):683–688
30. Russell AP, Hesselink MK, Lo SK, Schrauwen P (2005) Regulation of metabolic transcriptional co-activators and transcription factors with acute exercise. FASEB J 19(8):986–988. doi:10.1096/fj.04-3168fje
31. Watt MJ, Southgate RJ, Holmes AG, Febbraio MA (2004) Suppression of plasma free fatty acids upregulates peroxisome proliferator-activated receptor (PPAR) alpha and delta and PPAR coactivator 1alpha in human skeletal muscle, but not lipid regulatory genes. J Mol Endocrinol 33(2):533–544. doi:10.1677/jme.1.01499
32. Kang C, O'Moore KM, Dickman JR, Ji LL (2009) Exercise activation of muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha signaling is redox sensitive. Free Radic Biol Med 47(10):1394–1400. doi:10.1016/j.freeradbiomed.2009.08.007
33. Spangenburg EE, Brown DA, Johnson MS, Moore RL (2009) Alterations in peroxisome proliferator-activated receptor mRNA expression in skeletal muscle after acute and repeated bouts of exercise. Mol Cell Biochem 332(1–2):225–231. doi:10.1007/s11010-009-0195-1
34. Norrbom J, Sundberg CJ, Ameln H, Kraus WE, Jansson E (1985) Gustafsson T (2004) PGC-1alpha mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. J Appl Physiol 96(1):189–194. doi:10.1152/japplphysiol.00765.2003
35. Wang L, Sahlin K (2012) The effect of continuous and interval exercise on PGC-1alpha and PDK4 mRNA in type I and type II fibres of human skeletal muscle. Acta Physiol 204(4):525–532. doi:10.1111/j.1748-1716.2011.02354.x
36. Yeo WK, McGee SL, Carey AL, Paton CD, Garnham AP, Hargreaves M, Hawley JA (2010) Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen. Exp Physiol 95(2):351–358. doi:10.1113/expphysiol.2009.049353
37. Ambros V (2004) The functions of animal microRNAs. Nature 431(7006):350–355. doi:10.1038/nature02871
38. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297
39. Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, Yoshikawa T (2010) The microRNA miR-696 regulates PGC-1alpha in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab 298(4):E799–E806. doi:10.1152/ajpendo.00448.2009
40. Yamamoto H, Morino K, Nishio Y, Ugi S, Yoshizaki T, Kashiwagi A, Maegawa H (2012) MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead box j3. Am J Physiol Endocrinol Metab 303(12):E1419–E1427. doi:10.1152/ajpendo.00097.2012
41. Bori Z, Zhao Z, Koltai E, Fatouros IG, Jamurtas AZ, Douroudos II, Terzis G, Chatzinikolaou A, Sovatzidis A, Draganidis D, Boldogh I, Radak Z (2012) The effects of aging, physical training, and a single bout of exercise on mitochondrial protein

expression in human skeletal muscle. Exp Gerontol 47(6):417–424. doi:10.1016/j.exger.2012.03.004
42. Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH, Rosenzweig A, Spiegelman BM (2008) HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. Nature 451(7181):1008–1012. doi:10.1038/nature06613
43. Scarpulla RC (2008) Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol Rev 88(2):611–638. doi:10.1152/physrev.00025.2007
44. Dumke CL, Mark Davis J, Angela Murphy E, Nieman DC, Carmichael MD, Quindry JC, Travis Triplett N, Utter AC, Gross Gowin SJ, Henson DA, McAnulty SR, McAnulty LS (2009) Successive bouts of cycling stimulates genes associated with mitochondrial biogenesis. Eur J Appl Physiol 107(4):419–427. doi:10.1007/s00421-009-1143-1
45. Smith BK, Mukai K, Lally JS, Maher AC, Gurd BJ, Heigenhauser GJ, Spriet LL, Holloway GP (2013) AMP-activated protein kinase is required for exercise-induced peroxisome proliferator-activated receptor co-activator 1 translocation to subsarcolemmal mitochondria in skeletal muscle. J Physiol 591(Pt 6):1551–1561. doi:10.1113/jphysiol.2012.245944
46. Stepto NK, Benziane B, Wadley GD, Chibalin AV, Canny BJ, Eynon N, McConell GK (2012) Short-term intensified cycle training alters acute and chronic responses of PGC1alpha and Cytochrome C oxidase IV to exercise in human skeletal muscle. PLoS One 7(12):e53080. doi:10.1371/journal.pone.0053080
47. Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL (2010) Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. J Physiol 588(Pt 23):4795–4810. doi:10.1113/jphysiol.2010.199448
48. Pilegaard H, Saltin B, Neufer PD (2003) Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol 546(Pt 3):851–858
49. Safdar A, Little JP, Stokl AJ, Hettinga BP, Akhtar M, Tarnopolsky MA (2011) Exercise increases mitochondrial PGC-1alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. J Biol Chem 286(12):10605–10617. doi:10.1074/jbc.M110.211466
50. Daussin FN, Rasseneur L, Bouitbir J, Charles AL, Dufour SP, Geny B, Burelle Y, Richard R (2012) Different timing of changes in mitochondrial functions following endurance training. Med Sci Sports Exerc 44(2):217–224. doi:10.1249/MSS.0b013e31822b0bd4
51. Popov DV, Zinovkin RA, Karger EM, Tarasova OS, Vinogradova OL (2013) The effect of aerobic exercise on the expression of genes in skeletal muscles of trained and untrained men. Hum Physiol 39(2):190–195
52. Ljubicic V, Joseph AM, Saleem A, Uguccioni G, Collu-Marchese M, Lai RY, Nguyen LM, Hood DA (2010) Transcriptional and post-transcriptional regulation of mitochondrial biogenesis in skeletal muscle: effects of exercise and aging. Biochim Biophys Acta 3:223–234. doi:10.1016/j.bbagen.2009.07.031
53. Slivka D, Heesch M, Dumke C, Cuddy J, Hailes W, Ruby B (2013) Effects of post-exercise recovery in a cold environment on muscle glycogen, PGC-1alpha, and downstream transcription factors. Cryobiology 66(3):250–255. doi:10.1016/j.cryobiol.2013.02.005
54. Bishop DJ, Granata C, Eynon N (2014) Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content? Biochim Biophys Acta 4:1266–1275. doi:10.1016/j.bbagen.2013.10.012
55. Mazzatti DJ, Smith MA, Oita RC, Lim FL, White AJ, Reid MB (2008) Muscle unloading-induced metabolic remodeling is associated with acute alterations in PPARdelta and UCP-3 expression. Physiol Genomics 34(2):149–161. doi:10.1152/physiolgenomics.00281.2007
56. de Lange P, Farina P, Moreno M, Ragni M, Lombardi A, Silvestri E, Burrone L, Lanni A, Goglia F (2006) Sequential changes in the signal transduction responses of skeletal muscle following food deprivation. FASEB J 20(14):2579–2581. doi:10.1096/fj.06-6025fje
57. Mahoney DJ, Parise G, Melov S, Safdar A, Tarnopolsky MA (2005) Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise. FASEB J 19(11):1498–1500. doi:10.1096/fj.04-3149fje
58. Ehrenborg E, Krook A (2009) Regulation of skeletal muscle physiology and metabolism by peroxisome proliferator-activated receptor delta. Pharmacol Rev 61(3):373–393. doi:10.1124/pr.109.001560
59. Nemoto S, Fergusson MM, Finkel T (2005) SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1alpha. J Biol Chem 280(16):16456–16460. doi:10.1074/jbc.M501485200
60. Aquilano K, Vigilanza P, Baldelli S, Pagliei B, Rotilio G, Ciriolo MR (2010) Peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. J Biol Chem 285(28):21590–21599. doi:10.1074/jbc.M109.070169
61. Gurd BJ, Yoshida Y, McFarlan JT, Holloway GP, Moyes CD, Heigenhauser GJ, Spriet L, Bonen A (2011) Nuclear SIRT1 activity, but not protein content, regulates mitochondrial biogenesis in rat and human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 301(1):R67–R75. doi:10.1152/ajpregu.00417.2010
62. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD + metabolism and SIRT1 activity. Nature 458(7241):1056–1060. doi:10.1038/nature07813
63. Pardo PS, Mohamed JS, Lopez MA, Boriek AM (2011) Induction of Sirt1 by mechanical stretch of skeletal muscle through the early response factor EGR1 triggers an antioxidative response. J Biol Chem 286(4):2559–2566. doi:10.1074/jbc.M110.149153
64. Handschin C, Spiegelman BM (2008) The role of exercise and PGC1alpha in inflammation and chronic disease. Nature 454(7203):463–469. doi:10.1038/nature07206
65. Moflehi D, Kok LY, Tengku-Kamalden TF, Amri S (2012) Effect of single-session aerobic exercise with varying intensities on lipid peroxidation and muscle-damage markers in sedentary males. Glob J Health Sci 4(4):48–54. doi:10.5539/gjhs.v4n4p48
66. Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO (2007) Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1alpha expression. J Biol Chem 282(1):194–199. doi:10.1074/jbc.M606116200