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ORIGINAL PAPER

Exercise-induced expression of peroxisome proliferator-activated receptor γ coactivator-1α isoforms in skeletal muscle of endurance-trained males

Daniil V. Popov · Anton V. Bachinin · Evgeny A. Lysenko · Tatiana F. Miller · Olga L. Vinogradova

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Abstract The aim of the present study was to investigate the effect of acute aerobic exercise on the expression of PGC-1α transcript variants in human skeletal muscle. Seven endurance-trained athletes performed a 90-min cycling test (62 % of $\dot{V}o_{2max}$). At resting state, the levels of N-truncated (NT)-PGC-1α and PGC-1α exon 1a-derived transcripts were significantly higher (>20-fold; $P < 0.05$) than those of PGC-1α exon 1b- and 1c-derived transcripts. Acute exercise did not change the PGC-1α exon 1a-derived expression level, but it did increase the expression level of NT-PGC-1α mRNAs 6-fold, and the expression levels of PGC-1α exon 1b- and 1c-derived mRNAs >200-fold ($P < 0.05$). We conclude that NT-PGC-1α transcript expression in resting muscle and after acute moderate-intensity exercise constituted a significant share of total PGC-1α expression. The exercise led to a higher level of PGC-1α expression from alternative promoters (exon 1b- and 1c-derived mRNA) than from the canonical proximal promoter (exon 1a-derived mRNA).

Keywords Exercise · Skeletal muscle · PGC-1α gene · Alternative promoter · Gene expression

Introduction

Peroxisome proliferator-activated receptor gamma (PPARG) coactivator 1 alpha (PGC-1α) is a master regulator of mitochondrial biogenesis in the skeletal muscle. Endurance exercise induces an activation of AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase [1–3]. These kinases regulate the transcriptional initiation of the *PGC-1α* promoter as well as the phosphorylation of PGC-1α. Activated PGC-1α transits into the nucleus, resulting in the coactivation of transcription factors and an increase in the mRNA expression of genes involved in mitochondrial function and metabolism (e.g., citrate synthase, cytochrome *c* oxidase, β-hydroxyacyl-CoA dehydrogenase, and pyruvate dehydrogenase kinase-4) [4–6]. Moreover, activated PGC-1α can stimulate its own transcription [7].

The mRNA expression level of PGC-1α has been shown to be upregulated in human skeletal muscle after a single session of aerobic exercise, peaking 3–5 h after the termination of exercise [8, 9]. There are several mRNA transcript variants of PGC-1α in the skeletal muscle. The PGC-1α-a isoform (797 aa) is transcribed from the canonical proximal promoter, while PGC-1α-b and -c are transcribed from an alternative promoter that is ~14 kb upstream of the canonical one. The N-terminal regions of PGC-1α-b and -c are shorter than that of PGC-1α-a by 4 and 13 aa, respectively. The nucleotide sequences between exon 2 and exon 13 are the same in PGC-1α-a, -b, and -c mRNA. Studies in rodents have shown acute aerobic exercise to increase the mRNA expression of PGC-1α-a, -b and -c [10].

In an early study, Baar et al. showed a small PGC-1α mRNA transcript and protein (~34 kDa) to exist in rat skeletal muscle [11]. Zang and coworkers showed that alternative splicing of *PGC-1α* between exons 6 and 7 introduces an in-frame stop codon in new exon 7a and produces an additional transcript that codes for a short N-truncated (NT) PGC-1α isoform (~35–38 kDa, 270 aa) [12]. In mouse brown adipose tissue, the NT-PGC-1α

D. V. Popov (✉) · A. V. Bachinin · E. A. Lysenko · T. F. Miller · O. L. Vinogradova
Institute of Biomedical Problems, Russian Academy of Sciences, 76A Khoroshevskoe Shosse, Moscow 123007, Russia
e-mail: danil-popov@yandex.ru

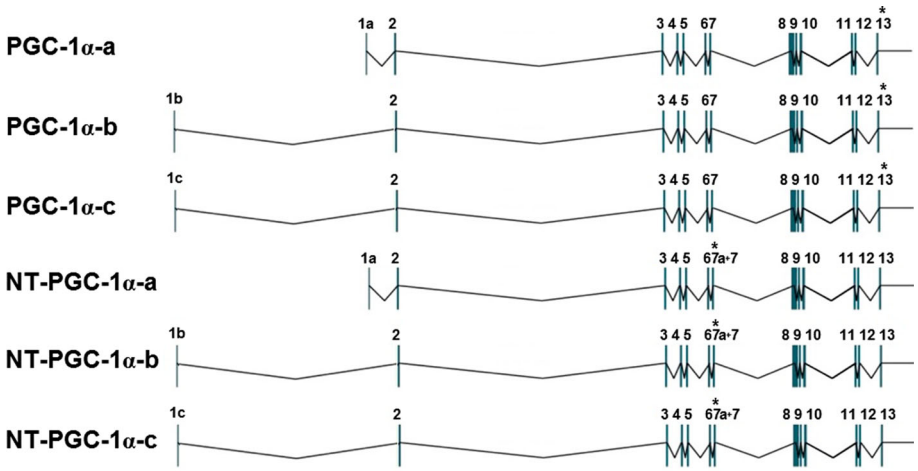

Fig. 1 Structure of different PGC-1α isoforms mRNA. Exon numbers are marked. *Asterisks* indicate stop codons

isoform can be transcribed from either the proximal (exon 1a-derived) or the alternative (exon 1b- and 1c-derived) promoter (Fig. 1) [13]. NT-PGC-1α isoforms have been found in mouse brain and kidney and human heart tissue [12].

Compared to full-length PGC-1α, the structure of the NT-PGC-1α protein specifies distinct patterns of subcellular localization, protein stability, transcription factor interaction, and target gene activation [12, 14, 15]. The NT-PGC-1α as opposed to full-length PGC-1α may activate a different transcription program. The truncated forms of PGC-1α strongly induce vascular endothelial growth factor expression and angiogenesis, and have little effect on mitochondrial genes [16]. Additionally, Ruas and coworkers reported an increase in the expression of NT-PGC-1α-b mRNA (PGC-1α4 in Ruas et al.'s study) and protein during skeletal muscle hypertrophy in mice [17].

To our knowledge, no investigations of NT-PGC-1α mRNA expression in human skeletal muscle after acute aerobic exercise have been done up till now, and only one study has investigated the effect of aerobic exercise on the mRNA expression of PGC-1α-a and -b in human skeletal muscle [18]. The goal of the present study was to investigate the effect of acute aerobic exercise on the expression levels of NT-PGC-1α mRNA, PGC-1α mRNA transcribed from the proximal (exon 1a-derived) promoter, PGC-1α mRNA transcribed from the alternative (exon 1b and 1c-derived) promoter, and total PGC-1α mRNA in human skeletal muscle. In a previous study, the elevated mRNA expression of PGC-1α in the skeletal muscle of untrained humans after acute aerobic exercise gradually decreased after 2 weeks of training, despite a continual increase in workload [19], illustrating that the mRNA expression of PGC-1α in untrained subjects after the first exercise session may be higher than after the next one. This is the reason behind our interest in investigating the expression of *PGC-1α* in the skeletal muscle of males adapted to endurance training. The second goal of the present study was to evaluate the relationship between mRNA expression of PGC-1α isoforms with the activation of AMPK and p38.

Materials and methods

Ethical approval

The study was approved by the Human Ethics Committee of the Institute of Biomedical Problems. All the participants had given their written consent to participate in this study. The study complied with the guidelines set forth in the Declaration of Helsinki.

Initial study

Seven amateur endurance-trained males [runners, cyclists, and cross-country skiers with a median weight of 70 (interquartile, 63–75) kg, height of 1.79 (1.77–1.82) m, and $\dot{V}o_{2max}$ of 66 (60–70) mL/min/kg body weight] participated in this study. During the first visit to the laboratory, the subjects were familiarized with the test. Two days later, each subject completed an incremental ramp test on the Ergoselect 200 electromagnetic bicycle ergometer (Ergoline, Germany). The initial load, load increment, and revolution rate were 0 W, 15 W/min, and 60 rpm, respectively. Each subject exercised until exhaustion. A revolution rate that slowed to 50 rpm and a respiratory exchange ratio that increased to more than 1.1 were indicative of exhaustion. The pulmonary oxygen uptake rate ($\dot{V}o_2$) was measured at consecutive 20-s intervals during the test using the AMIS 2000 medical mass-spectrometer with a mixing chamber (Innovision, Denmark). The highest $\dot{V}o_2$ value was taken as $\dot{V}o_{2max}$.

Primary study

All subjects were instructed to refrain from vigorous aerobic and strength exercises for 1 week, and to refrain from all exercises 36 h before the test. The subjects arrived at the laboratory at 0830 hours and had a standard breakfast (3,624 kJ; 24 g of protein, 157 g of carbohydrates, and 15 g of lipids). The exercise session started 1 h 45 min after breakfast, and consisted of a warm up (5 min, 50 % $\dot{V}o_{2max}$) and exercise (85 min, 60 % $\dot{V}o_{2max}$) sessions. The subjects had a standard lunch (3,650 kJ; 29 g of protein, 116 g of carbohydrates, and 43 g of lipids) 30 min after the termination of the exercise. Blood was drawn from fingertip capillaries prior to and every 15 min during exercise for determination of lactate concentration (Super GL Easy analyzer; Dr. Mueller Geraetebau, Germany). The serum concentration of nonesterified fatty acid (NEFA) was evaluated prior to and at 30, 60, and 90 min of exercise using the NEFA FS kit (DiaSys Diagnostic Systems, Germany). Biopsies were taken from the *m. vastus lateralis* using the microbiopsy technique [20] prior to and at 10 mi and, 3 and 5 h after exercise under local anesthesia (2 mL of 2 % lidocaine). The muscle samples were quickly blotted with a gauze to remove superficial blood, frozen in liquid N_2 for 20 s, and stored at −80 °C until analysis. The first biopsy was taken 12 cm proximal to the *condylus lateralis ossis femori*. Subsequent biopsies were taken 2 cm proximal to the previous one.

RNA extraction

RNA was extracted from ~20 mg wet muscle using the RNeasy Mini kit (Qiagen, Germany). The RNA concentration was measured by spectrophotometry (SmartSpec Plus; Bio-Rad, USA) at an absorbance of 260 nm, and RNA purity was assessed by the A260/A280 nm absorption ratio. After DNase treatment (Fermentas, Lithuania), cDNAs were obtained by annealing 1 μg of denatured (70 °C for 5 min) total RNA with oligo $(dT)_{15}$ at 40 °C for 60 min (Sileks, Russia).

Real-time PCR

Real-time PCR was carried out using the Rotor-Gene Q cycler (Qiagen). The annealing temperature for each primer set was optimized in trial PCR runs. The thermal profile included an initial heat-denaturing step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing (56 °C for *PGC-1α* isoforms and ribosomal protein, large, P0 (*RPLP0*), and 60 °C for actin, beta (*ACTB*) for 30 s, and extension at 72 °C for 30 s. Amplified genes were quantified by fluorescence using the EvaGreen® master mix (Syntol, Russia). Following amplification, the specificity of the amplification was monitored by the melting curves and by agarose gel (1 %) electrophoresis. Each sample was run in triplicate, and a nontemplate control was included in each run. Target gene mRNA expression level was calculated by the efficiency-corrected ΔC_t method as $(1 + E_{ref})^{Ct\ ref}/(1 + E_{tar})^{Ct\ tar}$, where C_t is the threshold cycle and E the PCR efficiency. E was calculated using standard curves corresponding to reference and target genes. Forward and reverse primers for detecting total PGC-1α were located in exon 2. Forward primers for detecting PGC-1α exons 1a-, 1b-, and 1c-derived transcripts were located in exons 1a, 1b, and 1c, respectively. All of the reverse primers were located in exon 2. It should be noted that these primers resulted in PCR amplification from both PGC-1α and NT-PGC-1α transcripts (Fig. 1). Forward primer and reverse primer for detecting all NT-PGC-1α isoforms were located in exon 5

Table 1 Primers used in this study

Transcript	Strand	Sequence, 5′–3′	Product size (bp)
Total PGC-1α	Forward	CAGCCTCTTTGCCCAGATCTT	101
	Reverse	TCACTGCACCACTTGAGTCCAC	
PGC-1α exon 1a-derived	Forward	ATG GAGTGACATCGAGTGTGCT	127
	Reverse	GAGTCCACCCAGAAAGCTGT	
PGC-1α exon 1b-derived	Forward	ATGAATGACACACATGTTGGG	156
	Reverse	AGTCCACCCAGAAAGCTGTCT	
PGC-1α exon 1c-derived	Forward	CTGCACCTAGGAGGCTTTATGC	138
	Reverse	CAATCCACCCAGAAAGCTGTCT	
NT-PGC-1α	Forward	TCACACCAAACCCACAGAGA	172
	Reverse	CTGGAAGATATGGCACAT	
RPLP0	Forward	CACTGAGATCAGGGACATGTTG	76
	Reverse	CTTCACATGGGGCAATGG	
ACTB	Forward	CGTGACATTAAGGAGAAGCTGTGC	374
	Reverse	CTCAGGAGGAGCAATGATCTTGAT	

and exon 7a, respectively. The primer sequences are shown in Table 1. The expression of both reference genes was unchanged after exercise.

Western blot

Frozen samples (~10 mg) were sectioned at 20 μm by a Leica ultratom (Germany) and homogenized in ice-cold RIPA buffer containing protease and phosphatase inhibitors (50 mM β-glycerophosphatase, 50 mM NaF, 1 mM Na_3VO_4, 20 μg/mL aprotinin, 50 μg/mL leupeptin, 20 μg/mL pepstatin, and 1 mM PMSF). Samples were then centrifuged for 10 min at 10,000*g* and 4 °C. Protein content was analyzed by the BCA assay. The samples (20 μg protein per lane) were mixed with Laemmli buffer, loaded onto a 10 % *T* polyacrylamide gel, and electrophoresis was performed in the Mini-Protean Tetra Cell system (Bio-Rad) at 20 mA per gel. The proteins were transferred onto nitrocellulose membrane using the Mini Trans-Blot system (Bio-Rad) in Towbin buffer for 3 h at 300 mA. The membrane was stained with Ponceau S to verify consistent loading of proteins, followed by washing and incubation in 5 % nonfat dried milk for 1 h. The membrane was then incubated with anti-phospho-AMPKα1/2 Thr^{172} (1:200; Santa Cruz Biotechnology, Germany), anti-phospho-p38 Thr^{180}/Tyr^{182} (1:500; Abcam, UK), or anti-β-actin (1:1,000 dilution; Santa Cruz Biotechnology) overnight at 4 °C. On the next day, the membrane was incubated with anti-rabbit secondary antibody (Cell Signaling, USA) for 1 h. After each step, the membrane was washed with PBS-Tween® 20 (3 times at 5 min each). The membrane was incubated with ECL substrate (Bio-Rad), luminescent signals were captured with X-ray film (Kodak, USA), and band intensities were densitometrically scanned with ImageJ software (National Institutes of Health, USA).

Statistics

In our study, the sample distributions were not normal, so the data were expressed as the median and interquartile range. Friedman one-way analysis of variance and post hoc Wilcoxon matched pairs signed-rank test with Bonferroni correction were used at a level of significance of 0.05.

Results

The average $\dot{V}o_2$ during cycling was 62 % (57–66 %) of $\dot{V}o_{2max}$. There was no change in the concentration of blood lactate during exercise. The NEFA level was 2-fold higher after a 90-min cycling session than the initial level at 0 min (Fig. 2).

At resting state, the expression of the PGC-1α exon 1a-derived transcript and the NT-PGC-1α transcript were identical. The levels of both transcripts were higher (>20-fold) than that of PGC-1α exon 1b-derived mRNA. The expression of PGC-1α exon 1c-derived mRNA was not detectable at resting state (Fig. 3). Acute aerobic exercise did not change the mRNA expression of the PGC-1α exon 1a-derived transcript, but it led to a marked increase

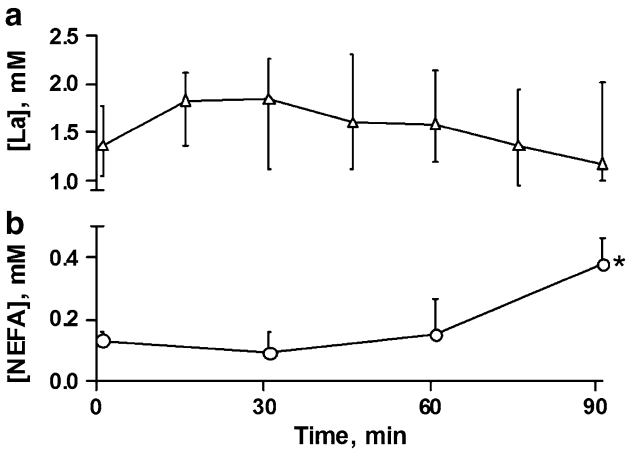

Fig. 2 Concentrations of capillary blood lactate (*La*; **a**) and nonesterified fatty acids (*NEFA*; **b**) during a 90-min cycling session (62 % of $\dot{V}o_{2max}$). *Asterisks* statistically different from the initial level at 0 min ($P < 0.05$). *Each value* represents the median and interquartile range

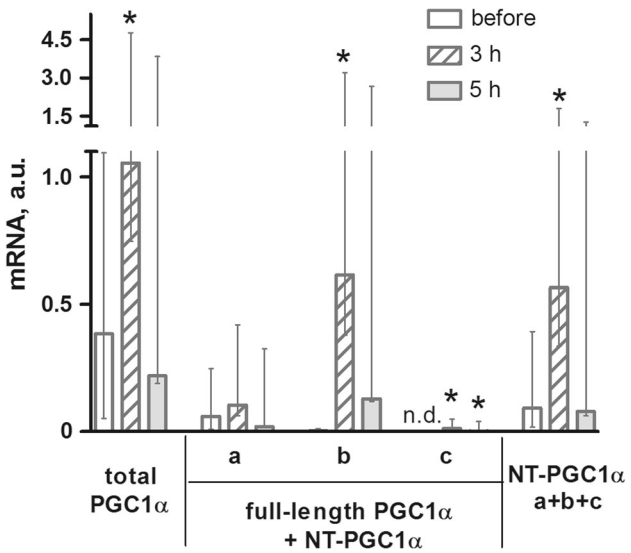

Fig. 3 Expression of total PGC-1α mRNA, PGC-1α exon 1a-, 1b-, and 1c-derived transcripts and NT-PGC-1α transcripts in *m. vastus lateralis* before and at 3 and 5 h after a 90-min cycling session (62 % of $\dot{V}o_{2max}$). At resting state, the expression of PGC-1α exon 1c-derived transcripts was not detectable (*n.d.*). *Asterisks* statistically different from the baseline level ($P < 0.05$). *Each value* represents the median and interquartile range

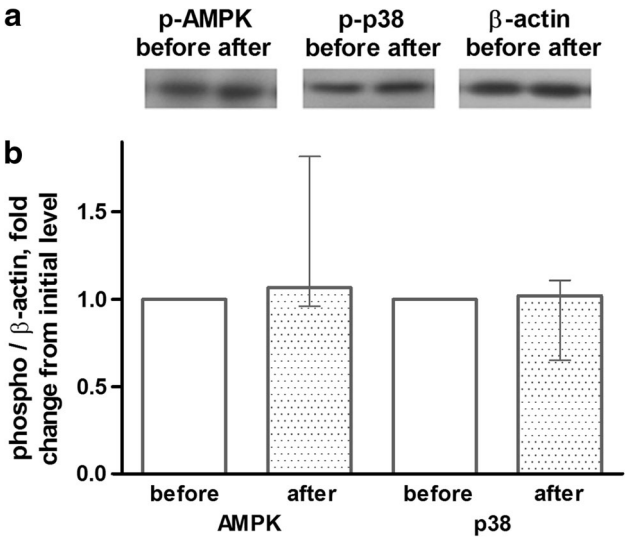

Fig. 4 Representative immunoblots (**a**) and quantification of AMPK and p38 phosphorylation levels (**b**) in *m. vastus lateralis* before and 10 min after a 90-min cycling session (62 % of $\dot{V}o_{2max}$). *Each value* represents the median and interquartile range

(>200-fold) in the expression of PGC-1α exon 1b and 1c-derived transcripts from 3 h after the termination of exercise (Fig. 3). During this period, ~50 % of total PGC-1α expression was constituted by PGC-1α exon 1b-derived mRNA. Exercise recovery resulted in a six-fold increase in NT-PGC-1α transcript expression.

There were no changes in the levels of phosphorylated p38 and AMPK 10 min after the termination of the exercise (Fig. 4). No significant correlations were found between the changes of p38 and AMPK phosphorylation levels and PGC-1α mRNA isoforms abundance.

Discussion

Our results have shown for the first time that the expression of NT-PGC-1α transcripts and PGC-1α exon 1a-derived transcripts was higher and that the expression of PGC-1α exon 1c-derived transcripts was lower in resting human skeletal muscle of endurance-trained males than the expression of PGC-1α exon 1b-derived mRNA. These findings are in agreement with data from Miura et al. [21] and Tadaishi et al. [10], who showed PGC-1α exon 1c-derived expression to be lower than that of PGC-1α exon 1b-derived, and the expression of PGC-1α exon 1b-derived mRNA to be several-fold lower than that of PGC-1α exon 1a-derived mRNA in the skeletal muscle of sedentary mice.

We found that a single exercise with low metabolic stress led to a 6-fold increase in the expression of all NT-PGC-1α transcripts during recovery. Moreover, the NT-PGC-1α transcripts constituted ~50 % of total PGC-1α expression at 3–5 h after the termination of the exercise. The increased NT-PGC-1α mRNA abundance might be favorable for endurance exercise-induced adaptation of human skeletal muscle, since NT-PGC-1α protein is relatively more stable than full-length protein due to lack of the C-terminal domain involved in proteosomal targeting [12]. Moreover, NT-PGC-1α exon 1a-, 1b-, and 1c-derived transcripts are functionally active, and can initiate PPARs-dependent genes expression as well as full-length PGC-1α [12, 13]. At the same time, truncated PGC-1α demonstrates unique properties. The NT-PGC-1α induces intensive expression of vascular endothelial growth factor and angiogenesis, while having little effect on mitochondrial genes [16]. Moreover, the NT-PGC-1α isoform transcribed from exon 1b (PGC-1α4 in Ruas et al.'s study) was found to regulate skeletal muscle hypertrophy in mice [17]. We investigate the endurance exercise-induced increase of all NT-PGC-1α mRNA in human skeletal muscle for the first time. A previous study found no change in the basal expression of all NT-PGC-1α transcripts (primer pairs to exons 5 and 7a) in human skeletal muscle after 8 weeks of aerobic training [17]. The effects of long-lasting endurance training and of acute exercise session may differ. It is possible that each exercise session induces elevation of the NT-PGC-1α transcripts expression, but the basal expression level is not changed. Moreover, 8 weeks of endurance training may not be enough to increase the basal NT-PGC-1α transcripts expression.

We did not observe an increase in the expression of PGC-1α exon 1a-derived mRNA after a single session of aerobic exercise. This may be due to the relatively low intensity of the exercise (62 % of $\dot{V}o_{2max}$) and to low metabolic stress. Indeed, Tadaishi et al. [10] showing that, in a rodent, an increase in the expression of PGC-1α exon 1a-derived transcripts could be obtained only after high-intensity exercise. Moreover, Norrbom and coworkers [18] found that the exercise-induced PGC-1α exon 1a-derived mRNA expression depends on metabolic stress in human skeletal muscle.

A single session of aerobic exercise in our study resulted in a >200-fold increase in the expression of PGC-1α exon 1b- and 1c-derived mRNA from 3 h after the termination of the exercise. This finding is in accordance with the marked increase in the expression of PGC-1α exon 1b- and 1c-derived transcripts in mouse skeletal muscle after 45 min of middle-intensity running [10, 21]. In our study, the PGC-1α exon 1b-derived mRNA constituted ~50 % of the total PGC-1α expression from 3–5 h after the termination of the exercise. Of note, its expression was identical to all NT transcripts. One may speculate that the increased PGC-1α exon 1b-derived transcript is NT-PGC-1α,

although this suggestion needs further confirmation. A significant increase in the expression of PGC-1α exon 1b-derived transcripts during recovery allows us to conclude that, in human skeletal muscle, the level of these mRNA isoforms is more sensitive to moderate-intensity exercise stimulus than the other isoforms. In our study, the total PGC-1α expression was higher compared with total expression of exon 1a-, 1b-, and 1c-derived isoforms, suggesting that isoforms derived from other exons might have existed. Such a possibility has been shown for human brain and liver [22, 23]. Recently, two additional skeletal muscle isoforms of PGC1α (PGC1α-2 and -3) were found in a rodent study [17]. Their functional role is unknown. It is likely that our primer pairs detected the PGC-1α-2 and -3 mRNA expressions as well as exon 1b- or 1c-derived mRNA expressions.

In our study, no significant changes were found in the levels of phosphorylated AMPK and p38, possibly because of low metabolic stress [24] and low intensity during exercise (62 % of $\dot{V}o_{2max}$). On the other hand, low metabolic stress during cycling might be related to a high fitness level of our subjects. While several subjects demonstrated an approximately 2-fold increase in the level of phosphorylated AMPK after the exercise (Fig. 4), there was no significant correlation between the expression of different PGC-1α isoforms and phosphorylated AMPK. For p38, no significant change was noted in its phosphorylation after exercise. We conclude that the moderate exercise-induced elevation of PGC-1α mRNA expression in trained skeletal muscle was independent of the activation of AMPK and p38. This result is supported by the results of studies in mice in which moderate-intensity exercise was found to induce the expression of PGC-1α exon 1b- and 1c-derived mRNAs mainly via the activation of β2-adrenergic receptors [25, 26]. By contrast, the increase of PGC-1α isoforms after high-intensity exercise was found to depend on multiple signaling pathways [10].

In conclusion, this study demonstrates that the expression of NT-PGC-1α transcripts in resting human skeletal muscle and after acute moderate-intensity aerobic exercise constitutes a half share of the total PGC-1α expression. Moderate-intensity aerobic exercise led to a higher level of PGC-1α expression from the alternative promoter (exon 1b- and 1c-derived mRNA) than that from the canonical proximal promoter (exon 1a-derived mRNA). Because of significant differences in the expression of different PGC-1α isoforms after acute exercise, future studies should investigate the expression of all mRNA and protein isoforms in human skeletal muscle.

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Conflict of interest The authors declare that they have no conflict of interest.

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