Rapidly elevated levels of PGC-1α-b protein in human skeletal muscle after exercise: exploring regulatory factors in a randomized controlled trial

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Rapidly elevated levels of PGC-1α-b protein in human skeletal muscle after exercise: exploring regulatory factors in a randomized controlled trial. J Appl Physiol 119: 374–384, 2015. First published June 18, 2015; doi:10.1152/japplphysiol.01000.2014. — Individuals with high skeletal muscle mitochondrial content have a lower risk to acquire cardiovascular and metabolic disease, obesity, and type II diabetes. Regular endurance training increases mitochondrial density through a complex network of transcriptional regulators that in an accumulated way are affected by each single exercise bout. The aim of the present study was to investigate the effect of a single exercise bout on the levels of PGC-1α-ex1b and related regulatory factors important for the initial phase of skeletal muscle adaptation. Ten men and ten women were randomized to either an exercise group (60 min cycling at a work load corresponding to 70% of peak oxygen uptake) or a nonexercising control group. Skeletal muscle biopsies were taken before, at 30 min, and at 2, 6, and 24 h after the intervention. Twenty-two mRNA transcripts and five proteins were measured. With exercise, protein levels of PGC-1α-ex1b increased, and this elevation occurred before that of total PGC-1α protein. We also demonstrated the existence and postexercise expression pattern of two LIPIN-1 (LIPIN-1α and LIPIN-1β) and three NCoR1 (NCoR1-1, NCoR1-2, and NCoR1-3) isoforms in human skeletal muscle. The present study contributes new insights into the initial signaling events following a single bout of exercise and emphasizes PGC-1α-ex1b as the most exercise-responsive PGC-1α isoform.

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REGULAR AEROBIC EXERCISE training is a strong inducer of mitochondrial biogenesis and improved oxidative capacity in skeletal muscle (24, 25). There is an inverse relationship between the dose of regular physical activity and the risk for premature death (7). This is in part explained by the mitochondrialrelated improvement of metabolic health in trained skeletal muscle (50, 57). Improved aerobic fitness and oxidative capacity have also been recognized to reduce the risk of morbidity and premature mortality in conditions such as cardiovascular and metabolic disease, obesity, and type II diabetes (12, 28). Thus low whole body aerobic capacity and muscle mitochondrial content are characteristics of a sedentary lifestyle that contribute to the development of metabolic disease and other disorders.

The morphology and functional properties of mitochondria are finely tuned to meet the energetic, metabolic, and signaling demands of the myocytes. Within the nucleus, an interconnected network of transcription factors and coregulators, i.e., coactivators and corepressors, control the expression of nuclear genes encoding mitochondrial proteins, including those controlling replication and transcription of the mitochondrial genome (55). Peroxisome proliferator-activated receptor (PPAR) gamma coactivators (PGCs) constitute a family of transcriptional coactivators proposed to be of significance for the regulation of mitochondrial biogenesis. PGCs coordinate the expression of numerous nuclear-encoded transcription factors, such as mitochondrial transcription factor-A (TFAM), nuclear respiratory factor-1 (NRF-1), and estrogen-related receptor alpha (ERRα) and gamma (ERRγ) (1, 27, 65). PGC-1α has been shown to be induced and activated in response to exercise in animal models and in humans (3, 10, 17, 39, 45).

There are four known PGC-1α isoforms, and it has been demonstrated that an alternative murine PGC-1α isoform, PGC-1α-ex1b, is transcribed from an upstream alternative promoter and that this transcript is greatly induced by exercise (36). We, and others, have also shown that several human PGC-1α mRNA transcripts are induced in response to both resistance and aerobic exercise in human skeletal muscle (33, 38, 49, 67). It has been hypothesized that different types of exercise influence the expression of PGC-1α isoform variants differently, generating distinct biological responses, such as angiogenesis and hypertrophy (9, 49). For example, PGC-1α has been suggested to induce vascular endothelial growth factor A (VEGF-A) expression by coactivating ERRγ (1). VEGF-B, a VEGF-A protein homolog, is affected by exercise training and has been coupled to PGC-1α/ERRs regulation and the elevation of metabolic gene expression in skeletal muscle (6, 22, 37).

The lipid metabolism enzyme LIPIN-1 can act as a transcriptional coregulator binding directly to, e.g., PGC-1α, enhancing its function (29, 48). LIPIN-1 has been suggested to play a role in skeletal muscle mitochondrial adaptation to endurance exercise (23). Péterfy et al. (43) described two different isoforms of LIPIN-1 mRNA in mouse adipocytes, LIPIN-1a and LIPIN-1b (43). Expression of these isoforms has not, to our knowledge, been reported to exist in human skeletal muscle. Furthermore, tumor protein p53 (p53), known for its regulatory role in cell cycle arrest, apoptosis, and DNA repair, is involved in similar pathways as LIPIN-1 (4). Muscle contraction and metabolic stress induces phosphorylation of p53, leading to nuclear and mitochondrial translocation, and in turn to transcription of mitochondrial genes such as PGC-1α, TFAM, and LIPIN-1 (2, 53). In addition, p53 has been shown to be essential for exercise-induced changes in mouse skeletal muscle and share upstream regulators with PGC-1α (40, 52, 66).
53). This supports p53’s putative role in PGC-1α-facilitated muscle metabolism and remodeling.

In addition to coactivators, corepressors contribute to transcriptional regulation. Two examples of coregulators that counteract PGC-1α action are nuclear receptor interacting protein 1 (NRIPI), also known as RIP140 (54), and nuclear receptor coressor 1 (NCoR1) (41). Studies have shown that NCoR1 and RIP140 interact with proteins involved in the metabolic response to exercise and inflammation (16, 41, 66). The existence of the NCoR1 isoforms NCoR1 isoform 1 (NCoR1-1), NCoR1 isoform 2 (NCoR1-2), and NCoR1 isoform 3 (NCoR1-3) has not previously been documented in human skeletal muscle.

The transition to a more oxidative skeletal muscle phenotype is dependent on an intricate network of signaling and transcriptional events accumulated over a series of repeated sessions (42, 44). Mapping the molecular response after a single exercise bout provides markers of early adaptational processes. This is of scientific importance and essential for optimizing exercise regimens for both patients and athletes at all levels. Accordingly, the present study aimed to investigate the effect of a single exercise bout on the levels of PGC-1α and its surrounding molecular network. To capture exercise-induced expression fluctuations, a nonexercise control group was included, and repeated skeletal muscle sampling over a 24-h period was performed. Special emphasis was put on the existence and expression of PGC-1α-ex1b, and LIPIN-1 and NCoR1 isoforms, in human skeletal muscle.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden. All subjects gave their written informed consent before participating. The study conformed to the standards set by the Declaration of Helsinki.

Subjects and Exercise Protocol

Twenty healthy subjects, ten men and ten women, were included in the study. Their mean ± SD age, height, and weight, for men and women, were 25 ± 2.7 and 24 ± 2.8 yr, 182 ± 4.8 and 169 ± 6.5 cm, and 80 ± 6.6 and 64 ± 6.5 kg, respectively. Prior to the intervention, their peak oxygen uptake ($\text{VO}_{2\text{peak}}$) was determined using an incremental cycle ergometer test until exhaustion, and respiratory gases were continuously analyzed (Sensor Medics Vmax 229; Intra Medic AB, Bästa, Sweden). Subjects with a $\text{VO}_{2\text{peak}} < 60 \text{ml kg}^{-1}\text{min}^{-1}$ (men) and <50 $\text{ml kg}^{-1}\text{min}^{-1}$ (women) were included in the study, as an elite athlete training status might diminish the possibility of detecting significant exercise effects. Mean $\text{VO}_{2\text{peak}}$ was 50.2 ± 4.6 $\text{ml kg}^{-1}\text{min}^{-1}$ for men and 41.5 ± 3.6 $\text{ml kg}^{-1}\text{min}^{-1}$ for women.

After inclusion in the study, subjects were randomly assigned to either an exercise group (ExG), seven men and eight women, or a nonexercising control group (CG), three men and two women. There were no significant differences between the groups regarding age, height, weight, and $\text{VO}_{2\text{peak}}$. The ExG performed 60 min of cycling exercise at a workload corresponding to 70% of their $\text{VO}_{2\text{peak}}$, whereas the subjects in the CG were resting. Subjects rested between biopsies (described below) and stayed in the laboratory until after the 6-h postexercise biopsy and returned to the laboratory the next morning for the 24-h biopsy. To control for circadian rhythm effects, all subjects reported to the laboratory in the morning of the intervention. All subjects were given standardized meals the night before, during the day of, and on the morning after the intervention.

Biopsy Protocol

Muscle biopsies from the vastus lateralis muscle were obtained at rest before the exercise bout (Pre, from both legs), at 30 min, and at 2, 6, and 24 h after exercise using a percutaneous needle biopsy technique (5). All biopsy samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis. Biopsies were obtained from the left and right leg alternately and the incisions were proximal to the former and separated by at least 2 cm to minimize the potential effects of local inflammation.

RNA Extraction

Total RNA from the skeletal muscle biopsies was prepared (from ~15 mg of tissue) using an acid phenol method (11) and quantified spectrophotometrically by measuring absorbance at 260 nm (NanoDrop 2000; Thermo Scientific, Gothenburg, Sweden). Two micrograms of total RNA were reverse transcribed using Superscript reverse transcriptase (Life Technologies, Stockholm, Sweden) and random hexamer primers (Roche Diagnostics, Mannheim, Germany) in a total volume of 20 µl. Samples were stored at −80°C until further use.

Reverse Transcriptase PCR

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed with primers to detect LIPIN-1α, LIPIN-1β, and NCoR1 isoforms (NCoR1-1, NCoR1-2, and NCoR1-3) in human skeletal muscle. Primers were designed based on sequence homology of LIPIN-1 and NCoR1, respectively. The total RT-PCR reaction volume was 25 µl, including 5 µl of cDNA sample diluted 1:100; forward and reverse primers (final concentration 0.4 µmol/l); 250 nmol/l of deoxyribonucleotide triphosphates (dNTPs); and 2.5 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). The RT-PCR products were then run on an agarose gel.

Real-Time RT-PCR

Real-time RT-PCR was used for mRNA quantification of PGC-1α [total PGC-1α, PGC-1α-ex1a, PGC-1α-ex1b, trunca-PGC-1α, and non-trunc-PGC-1; see Ydfors et al. (67) for amplicon description], LIPIN-1, LIPIN-1α, and LIPIN-1β, NCoR1, NCoR1-1, NCoR1-2, and NCoR1-3. Primers were designed to cover exon-exon boundaries to avoid amplification of genomic DNA. All primers for detecting PGC-1α (38, 67), LIPIN-1 (Fig. 1A), and NCoR1 (Fig. 1B) mRNA and their isoforms, were synthesized by Cybergene AB, Stockholm, Sweden (for primer sequences, see Table 1). The total reaction volume was 15 µl, containing 5 µl cDNA sample; forward primer (final concentration 0.4 µM); reverse primer (final concentration 0.4 µM); and SYBR Green PCR Master Mix (Applied Biosystems). All quantification reactions were controlled with a melting curve, and primer efficiency was tested using standard curves. For quantification of the remaining factors, the following TaqMan Gene Expression Assays (Applied Biosystems) were used: protein kinase C epsilon (PKCe) (Hs00178455), ERα (Hs01067166), ERβ (Hs00976243), succinate dehydrogenase complex, subunit A (SDHA) (Hs00188166), VEGF-A (Hs09999670), VEGF-B (Hs00173634), NCoR1 (Hs01094540), p53 (Hs01034249), RIP140 (Hs00942766), NRF-1 (Hs00602161), TFAM (Hs00273372), mitochondrial transcription factor B1 (TFB1M) (Hs00274971), and LIPIN-1 (Hs00299515). The total reaction volume was 10 µl, containing 4.5 µl cDNA sample; 5 µl TaqMan Fast Universal PCR Master Mix (Applied Biosystems); and 0.5 µl gene-specific primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control (4352934E, Applied Biosystems) and was stable across all time points. All reactions were performed in 96-well MicroAmp Optical plates, with sample duplicates, using Applied Biosystems 7500 Fast Real-Time PCR System.
**Protein Extraction and Western Blot**

Muscle samples (~20 mg) were homogenized in RIPA buffer (20 μl RIPA/mg tissue) containing 150 mM NaCl, 10 mM Tris-HCl (pH 7.8), 5 mM EDTA (pH 7.4), 0.5% Na deoxycholate, 0.1% SDS, 1% Triton X-100, and 1× complete protease inhibitor cocktail (Roche Diagnostics), using glass homogenizers. The homogenate was gently rotated at 4°C for 60 min, followed by centrifugation at 4°C for 10 min (15,000 g). Samples were stored at -80°C until further use.

Skeletal muscle homogenates (20 μg protein/sample) were separated electrophoretically on 4-15% SDS-PAGE gels (BioRad, Stockholm, Sweden) and proteins were then blotted onto PVDF membranes (Millipore, Billerica, MA). The membranes were blocked for 1 h at room temperature in blocking reagent (Millipore) and then incubated with primary antibodies for PGC-1α/β (sc-20681) and PGC-1α/β-ex1b antibodies from Calbiochem/Aldrich, St. Louis, MO; T5201) was used as loading control. All antibodies were diluted in blocking reagent:ddH2O (1:1) and incubated overnight at 4°C, and 45 min at room temperature the day after. After washing with PBS-T (0.1% Tween 20), membranes were incubated for 1 h at room temperature with IRDye secondary antibody (LI-COR Biosciences, Cambridge, UK). Membranes were scanned using Odyssey SA Infrared Imaging System (LI-COR Biosciences), and quantified using ImageJ (National Institutes of Health, Bethesda, MA) or ImageStudio (LI-COR Biosciences).

**Nuclear and Cytoplasmic Extractions**

Nuclear and cytoplasmic fractions were prepared using the Nuclear and Cytoplasmic Extraction Kit (NE-PER Nuclear and Cytoplasmic Extraction Reagents, Thermo Scientific, 78833). In brief, 10–20 mg of muscle sample (ExG, n = 5) was washed twice with PBS and then homogenized in cytoplasmic extraction reagent (CER) buffer containing protease inhibitors (Complete Mini, Roche Diagnostics) using a handheld plastic homogenizer. The cytoplasmic fraction was then extracted using CER II buffer. Pellets containing the nuclear fraction were washed twice in PBS to remove contaminating cytoplasmic proteins before nuclear proteins were extracted in nuclear extraction reagent (NER) buffer. The purity of the fractions was tested using Western blot with antibodies specific for the nuclear protein Lamin A/C (1:200, Santa Cruz, sc-20681) and the cytoplasmic protein beta-tubulin.

**Statistical Analyses**

Gene expression and total protein abundance in response to a single bout of exercise were analyzed using linear mixed models (LMM) with
factors time (Pre and at 30 min, and 2, 6, and 24 h after exercise) × group (exercise and control), followed by pairwise comparison within groups using Fisher’s least significant difference (LSD). The statistical analyses for mRNA expression were performed using the ΔCt value obtained by subtracting the GAPDH Ct value from the respective target Ct value. Nuclear and cytoplasmic protein abundance was analyzed using one-way repeated-measures analysis of variance (ANOVA) to test time effects (change from baseline) in the exercise group. The level of significance was set at 5% (P < 0.05) for all statistical analyses. All statistical analyses were performed using SPSS version 22 (IBM SPSS, Chicago, IL). Outliers, defined as observations <Q1 – (1.5 × IQR) or >Q3 + (1.5 × IQR) were excluded from the analysis. Three subjects were excluded from the mRNA analysis of PGC-1α isoforms since they have been included in a previous report (67). Protein analyses could not be performed for three of the subjects in the ExG and for one subject in the CG due to sample limitations.

RESULTS

mRNA Levels of Transcriptional Activators After One Bout of Endurance Exercise

PGC-1α. The mRNA levels of Total PGC-1α, PGC-1α-ex1b, trunc-PGC-1α, and non-trunc-PGC-1α isoforms were significantly different between the ExG and the CG (P < 0.01; Fig. 2, A, B, D, and E). Total PGC-1α and PGC-1α-ex1b mRNA in the ExG were significantly increased at 30 min (2.4-fold, P < 0.01, and 468-fold, P < 0.01, respectively), 2 h (8.6-fold, P < 0.01, and 1,341-fold, P < 0.01, respectively), 6 h (5.7-fold, P < 0.01, and 1,003-fold, P < 0.01, respectively), and 24 h (1.7-fold, P = 0.01, and 14.8-fold, P < 0.01, respectively) compared with Pre. In the CG, total PGC-1α mRNA was significantly increased at 2 h (1.8-fold, P < 0.01) and 6 h (1.7-fold, P < 0.01) compared with Pre, and PGC-1α-ex1b mRNA increased significantly at 2 h (4.7-fold, P < 0.01) compared with Pre. Notably, PGC-1α-ex1b mRNA levels were very low (Ct > 30) before exercise.

In the ExG, trunc-PGC-1α and non-trunc-PGC-1α mRNA expression increased significantly at 30 min (2.4-fold, P < 0.01, and 2.2-fold, P = 0.01, respectively), 2 h (14.6-fold, P < 0.01, and 9.6-fold, P < 0.01, respectively), and 6 h (8.9-fold, P < 0.01, and 7.0-fold, P < 0.01, respectively) compared with Pre. Notably, PGC-1α-ex1a mRNA expression did not differ between the ExG and the CG (Fig. 2C). In the ExG, PGC-1α-ex1a mRNA was increased at 30 min (2.7-fold, P < 0.01), 2 h (3.2-fold, P < 0.01), and 6 h (2.1-fold, P < 0.01) compared with Pre. In the CG, PGC-1α-ex1a mRNA was significantly increased at 2 h (1.5-fold, P = 0.01) compared with Pre.
significantly increased at 2 h (1.7-fold, \( P < 0.01 \)) and 6 h (1.6-fold, \( P < 0.01 \)) compared with Pre.

**LIPIN-1.** Analyses of the RT-PCR products on the agarose gel demonstrate that at least two alternative LIPIN-1 transcripts exist in human skeletal muscle (Fig. 1C). As predicted from sequence alignments, total LIPIN-1 transcripts were detected at \( \sim 237 \) base pairs (bps) and \( \sim 345 \) bps. LIPIN-1\( \alpha \) was amplified using a reverse primer spanning exon 9–11, thus excluding exon 10 and generating a transcript at \( \sim 313 \) bps. There were significant differences between the ExG and the CG in total LIPIN-1 (\( P < 0.01 \); Fig. 3A) and LIPIN-1\( \alpha \) (\( P < 0.05 \); Fig. 3B) mRNA expression. In the ExG, total LIPIN-1 mRNA expression was significantly increased at 2 h (1.7-fold, \( P < 0.01 \)), 6 h (2.1-fold, \( P < 0.01 \)), and 24 h (1.9-fold, \( P < 0.01 \)) compared with Pre. Total LIPIN-1\( \alpha \) mRNA expression was significantly increased at 30 min (1.4-fold, \( P = 0.05 \)), 6 h (1.8-fold, \( P < 0.01 \)), and 24 h (1.9-fold, \( P < 0.01 \)) compared with Pre. In the CG, LIPIN-1\( \alpha \) mRNA expression was significantly higher at 2 h (1.4-fold, \( P < 0.05 \)) compared with Pre. Also, both total LIPIN-1 and LIPIN-1\( \alpha \) mRNA expression were significantly higher at 24 h (1.9-fold, \( P < 0.01 \), for both) compared with Pre. There was no significant difference in LIPIN-1\( \beta \) mRNA expression between the ExG and the CG (Fig. 3C). In the ExG, LIPIN-1\( \beta \) mRNA expression was significantly increased at 6 h (2.7-fold, \( P < 0.01 \)) and at 24 h (1.9-fold, \( P < 0.01 \)) compared with Pre. In the CG, LIPIN-1\( \beta \) mRNA expression was significantly higher at 6 h (1.6-fold, \( P < 0.01 \)) and at 24 h (1.8-fold, \( P < 0.01 \)) compared with Pre.

ERR\( \alpha \), ERR\( \gamma \), p53, NRF-1, TFAM, VEGF-A, and VEGF-B. There was no significant difference in ERR\( \alpha \) mRNA levels, either between groups or over time within the two groups (Fig. 4A). The mRNA levels of ERR\( \gamma \) were significantly between the ExG and the CG (\( P < 0.05 \); Fig. 4B). In the ExG, there was a significant increase at 6 h (5.1-fold, \( P < 0.01 \)).

p53 mRNA expression was significantly different between the ExG and the CG (\( P < 0.05 \); Fig. 4C). p53 mRNA expression was significantly increased at all time points after exercise (30 min: 1.7-fold, \( P = 0.01 \); 2 h: 1.6-fold, \( P = 0.05 \); 6 h: 2.9-fold, \( P < 0.01 \); 24 h: 1.7-fold, \( P = 0.01 \)). p53 mRNA expression increased significantly in the CG at all time points, except for 6 h (30 min: 1.7-fold, \( P < 0.05 \); 2 h: 1.7-fold, \( P < 0.05 \); 24 h: 1.9-fold, \( P < 0.01 \)).

NRF-1 mRNA levels did not differ between the ExG and the CG (Fig. 4D). However, there was a significant increase in the CG at 6 h (1.4-fold, \( P < 0.05 \)) and at 24 h (1.5-fold, \( P = 0.01 \)) compared with Pre.

The mRNA levels of TFAM were significantly different between the ExG and the CG (\( P < 0.05 \); Fig. 4E). In the ExG, the TFAM mRNA expression was significantly increased at 30 min (1.4-fold, \( P = 0.01 \)) and 6 h (1.4-fold, \( P < 0.01 \)) compared with Pre.

The VEGF-A mRNA expression was significantly different compared with the ExG and the CG (\( P < 0.05 \); Fig. 5A). VEGF-A mRNA increased significantly at 30 min (2.7-fold, \( P < 0.01 \)), 2 h (3.8-fold, \( P < 0.01 \)), and 6 h (3.4-fold, \( P < 0.01 \)) in the ExG compared with Pre. The expression of VEGF-B did not differ between the ExG and the CG (Fig. 5B). There was no significant change over time in the ExG. However, in the CG, there were small but significant differences at all time points (30 min: 1.1-fold, \( P < 0.05 \); 2 h: 1.2-fold, \( P = 0.01 \); 6 h: 1.2-fold, \( P < 0.01 \); 24 h: 1.1-fold, \( P < 0.05 \)) compared with Pre.

**PKCe, TFB1M, and SDHA.** The PKCe mRNA levels did not differ between the ExG and the CG (Fig. 5C). However, in the ExG, PKCe mRNA decreased significantly at 2 h (0.9-fold, \( P < 0.05 \)) and even further at 24 h (0.8-fold, \( P < 0.01 \)) compared with Pre.

The TFB1M mRNA levels did not differ between the ExG and the CG. TFB1M mRNA levels were significantly elevated at 24 h compared with Pre in the CG (1.5-fold, \( P < 0.01 \); Fig. 5D). SDHA mRNA expression did not differ between the ExG and the CG, nor over time within the groups (Fig. 5E).

**mRNA levels of Transcriptional Repressors After One Bout of Endurance Exercise**

**RIP140.** RIP140 mRNA levels were not significantly different between the ExG and the CG (\( P = 0.06 \); Fig. 6A). However, one bout of cycling exercise significantly increased RIP140 mRNA expression at all time points (30 min: 1.5-fold, \( P < 0.05 \); 2 h: 1.7-fold, \( P < 0.01 \); 6 h: 3.4-fold, \( P < 0.01 \); 24 h: 1.7-fold, \( P < 0.01 \)) compared with Pre.

**NCoRI.** Analyses of the RT-PCR products on the agarose gel demonstrate that at least three alternative NCoRI transcripts exist in human skeletal muscle (Fig. 1D). As predicted from sequence alignments, we visualized NCoRI-1 at \( \sim 157 \) bps, NCoRI-2 at \( \sim 84 \) bps, and NCoRI-3 at \( \sim 214 \) bps.

The postexercise mRNA levels of NCoRI-1, NCoRI-2, and NCoRI-3 were not significantly different between the ExG and the CG (Fig. 6, B–E). In the ExG, NCoRI mRNA expression

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**Fig. 3.** mRNA levels of total LIPIN-1 (A), LIPIN-1\( \alpha \) (B), and LIPIN-1\( \beta \) (C) in human skeletal muscle after one bout of endurance cycling; \( n = 15 \) in the exercise and control group, respectively. \(*P \leq 0.05 \) with factors time \( \times \) group (LMM). \(*P \leq 0.05, **P \leq 0.01 \) compared with Pre [pairwise comparison (LSD) within groups]. Values are presented as means ± SE.
was significantly increased at 30 min compared with Pre (1.8-fold, \( P < 0.05 \)). Also, the NCoR1-3 mRNA expression was significantly increased 24 h after exercise compared with Pre (1.7-fold, \( P < 0.01 \)). In the CG, NCoR1 mRNA expression was significantly increased at 6 h (1.5-fold, \( P < 0.05 \)) and at 24 h (1.9-fold, \( P < 0.01 \)) compared with Pre. Also, NCoR1-2 mRNA was significantly elevated at 24 h compared with Pre (2.5-fold, \( P < 0.05 \)).

**Total Protein Levels After One Bout of Endurance Exercise**

**PGC-1α.** Total PGC-1α protein levels were not significantly different between the two groups but were significantly increased at 24 h after exercise compared with Pre (1.2-fold, \( P < 0.05 \); Fig. 7A). PGC-1α-ex1b protein levels were not significantly different between the ExG and the CG but increased 3.1-fold at 30 min compared with Pre in the ExG (\( P = 0.05 \); Fig. 7B). Representative blots are shown in Fig. 7G. PKCe protein levels did not differ between the ExG and the CG. However, PKCe protein levels were significantly higher at 2 h (1.3-fold, \( P = 0.01 \)) in the ExG compared with Pre.

**RIP140 and PKCe.** RIP140 protein levels were significantly different between the ExG and the CG. In the ExG, RIP140 protein levels were significantly increased at 6 h (1.3-fold, \( P < 0.01 \)) compared with Pre. Interestingly, in the CG, RIP140 protein levels were significantly increased at 2 h (1.8-fold, \( P < 0.05 \)) and at 24 h (1.8-fold, \( P < 0.01 \)) compared with Pre (Fig. 7C). Representative blots are shown in Fig. 7G.

**Nuclear and Cytoplasmic Protein Levels Before and After Exercise**

Total PGC-1α and PGC-1α-ex1b protein levels did not change significantly over time in either the nuclear or the cytoplasmic fraction. RIP140 protein levels did not change significantly over time in the nuclear fraction and could not be detected in the cytoplasmic fraction (Fig. 7, D–F). Representative blots are shown in Fig. 7H.

**DISCUSSION**

In the present study, we explored the time series response of factors associated with oxidative adaptations in human skeletal muscle after an acute bout of exercise. By including a non-exercising control group this enables the possibility to identify true exercise effects.

For the first time, we establish the presence and the postexercise time pattern of PGC-1α-ex1b protein. The earlier peak of PGC-1α-ex1b than that of total PGC-1α may
imply that PGC-1α-ex1b contributes to an earlier phase of mitochondrial adaptation. This could explain the fact that mitochondrial genes, and genes associated with mitochondrial biogenesis, are activated before total PGC-1α protein (42, 64). A rapid mRNA translation into protein or increased posttranslational stabilization, resulting in a positive protein turnover, could explain the early concurrent elevation of PGC-1α-ex1b mRNA and protein. The increase in total PGC-1α mRNA and protein is consistent with previous studies (31, 38, 42). Our study provides the important insight that PGC-1α-ex1b protein expression is more responsive to exercise compared with other PGC-1α isoforms. However, in contrast to earlier findings (32) we could not observe a significant nuclear accumulation of PGC-1α protein after acute exercise.

All PGC-1α mRNA isoforms displayed a similar postexercise expression pattern but differed with regard to magnitude of change. This supports the notion that they are differently regulated and that the expression of each variant depends on, e.g., exercise type, duration, intensity, and frequency (33, 38, 63, 67). Also, several different stimuli can trigger the expression of PGC-1α, i.e., the induction is not solely dependent on exercise (10, 38, 46, 49), in the present study indicated by an induction in the control group.

The knowledge regarding LIPIN-1, a protein with the ability to bind PGC-1α, in skeletal muscle after exercise is limited. We show that two isoforms (LIPIN-1α and LIPIN-1β) exist in human skeletal muscle. Animal studies have indicated that LIPIN-1α is more abundant in the nucleus than its beta counterpart and therefore more probable to engage in transcriptional control (43). This might explain the difference between the LIPIN-1α and LIPIN-1β mRNA expression pattern in our study. We were not able to detect LIPIN-1 protein in our samples. However, since LIPIN-1 is known to be posttranslationally modulated and can dissociate the corepressor NCoR1 from PPARγ, initiating gene transcription (29), it is of importance to elucidate whether LIPIN-1 protein levels are affected by exercise.

We report that three different NCoR1 isoforms are expressed in human skeletal muscle. However, no major expressional changes were found in either the exercise or the control group. Still, it is possible that the isoforms have diverse functions in human skeletal muscle tissue, although not affected by acute exercise. NCoR1 protein studies would add interesting knowledge about the function of corepressors in the shift into a more adapted skeletal muscle. However, similar to Yamamoto et al. (66), we were unable to detect NCoR1 protein in our skeletal muscle samples.

mRNA levels of the PGC-1α antagonist RIP140 were clearly upregulated after exercise, which is consistent with findings in a previous study (15). Interestingly, protein levels of RIP140 were less induced in the ExG compared with the CG, which may suggest that muscle contraction abates the inhibitory effects of RIP140 on the oxidative metabolism.
machinery. It may be speculated that the blunted RIP140 protein response with exercise is due to the inhibitory actions of PGC-1α and ERRγ (8, 54). Based on observations of PKCε-facilitated RIP140 nuclear export in adipocytes (18, 58) and earlier studies in skeletal muscle (26, 54), we studied the subcellular localization of RIP140. Interestingly, we could only detect RIP140 protein in the nuclear fraction. As RIP140 protein abundance in whole cell lysates differed between the two groups, studying this in the control group would have added important perspective on the RIP140 exercise response. However, due to limited amounts of material, this was not possible. To clarify the role of PKCε in RIP140 nuclear export regulation, future studies could include long-term exercise training and analysis of PKCε-mediated phosphorylation and subsequent methylation of RIP140.

Another suggested contributor to the exercise adaptation machinery is p53 (51). However, the acute mRNA response in this study does not further explain its role since there was an mRNA induction in both groups. Therefore it might be speculated that the biopsy procedure itself could stimulate p53 expression. A protein-protein interaction between p53 and TFAM has previously been suggested (40, 53); however, our TFAM mRNA data show a biphasic elevation that is not concomitant with the p53 expression. It may be speculated that the 30-min elevation of TFAM mRNA is induced by an immediate and transient increase of p53 phosphorylation whereas the second elevation might be more influenced by PGC-1α co-activation, together with, e.g., NRF-1 (3). Hence, TFAM is likely regulated in other ways than through p53 binding (4, 61).

As a marker of p53 action, we examined the expression of one of its established target genes, VEGF-A (14). Exercise significantly induced VEGF-A mRNA, supporting findings from others (19, 20). One argument against a predominant p53-mediated VEGF-A induction is the finding that the VEGF-A mRNA peak comes prior to the p53 peak. One explanation might be that biopsies per se can influence muscle oxygen status and thereby trigger expression of hypoxic-sensitive genes such as VEGF-A (60). Furthermore, PGC-1α has been proven to positively regulate VEGF-A transcription by coactivating ERRγ (59), perhaps in a p53-independent manner. No significant elevation in ERRγ mRNA levels could be established in the present study, although inductions by contractile activity have been reported previously (13, 62). These discrepancies might be explained by differences in exercise regimens and biopsy time points.

Contrary to what we hypothesized, the levels of VEGF-B mRNA did not change following acute exercise. Although PGC-1α has been suggested to be one of the inducers of VEGF-B mRNA (6, 21), transgenic mouse experiments indicate a PGC-1α-independent mechanism for stimulating
VEGF-B expression (37). This, together with the possibility of a later expression onset than 24 h postexercise, could explain our findings. Interestingly, in contrast to ERR\(\alpha\), ERR\(\gamma\) mRNA was significantly induced in the exercise group. ERR\(\gamma\) has been linked to activation of AMP-activated protein kinase (AMPK) and presumably important in promoting exercise-induced metabolic adaptations (37, 47). This might imply that ERR\(\gamma\) is more important as an acute exercise-response target gene than ERR\(\alpha\).

Repeated biopsies have been performed previously in both animal and human studies. However, it is common that these types of studies do not account for the possible diurnal changes or effects of inflammation that might occur. In contrast to most previous studies we included a nonexercising control group to isolate the exercise-specific effects. Thereby the impact of, e.g., biological variability, biopsy effects, and diurnal changes (30, 34, 35, 56, 60) are minimized in the analysis and interpretation of the data. This is reflected by the finding that some of the measured factors in the control group are still upregulated at 24 h in the present study. Thus this study contributes to the field of molecular exercise physiology.

In conclusion, the present study establishes that PGC-1\(\alpha\)-ex1b mRNA and protein levels are rapidly elevated by exercise and highlights PGC-1\(\alpha\)-ex1b as the most exercise-responsive PGC-1\(\alpha\) isoform. We also show the existence of two LIPIN-1 (LIPIN-1\(\alpha\) and LIPIN-1\(\beta\)) and three NCoR1 isoforms (NCoR1-1, NCoR1-2, and NCoR1-3) in human skeletal muscle. The current study demonstrates that RIP140 protein levels are lower in the exercising group, which prompts us to speculate that the inhibitory actions of RIP140 are suppressed. Our findings improve the understanding of early signaling events in response to exercise and emphasize the need to include a nonexercising control group.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


