Resistance exercise enhances the molecular signaling of mitochondrial biogenesis induced by endurance exercise in human skeletal muscle

Li Wang,¹ Henrik Mascher,¹,²* Niklas Psilander,¹,²* Eva Blomstrand,¹,² and Kent Sahlin¹,²

¹The Åstrand Laboratory of Work Physiology, GIH, the Swedish School of Sport and Health Sciences, and ²Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Submitted 21 January 2011; accepted in final form 9 August 2011

Wang L, Mascher H, Psilander N, Blomstrand E, Sahlin K. Resistance exercise enhances the molecular signaling of mitochondrial biogenesis induced by endurance exercise in human skeletal muscle. J Appl Physiol 111: 1335–1344, 2011. First published August 11, 2011; doi:10.1152/japplphysiol.00086.2011.—Combining endurance and strength training (concurrent training) may change the adaptation compared with single mode training. However, the site of interaction and the mechanisms are unclear. We have investigated the hypothesis that molecular signaling of mitochondrial biogenesis after endurance exercise is impaired by resistance exercise. Ten healthy subjects performed either only endurance exercise (E; 1-h cycling at ~65% of maximal oxygen uptake), or endurance exercise followed by resistance exercise (ER; 1-h cycling + 6 sets of leg press at 70–80% of 1 repetition maximum) in a randomized cross-over design. Muscle biopsies were obtained before and after exercise (1 and 3 h postexercise). The mRNA of genes related to mitochondrial biogenesis ([peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1)α, PGC-1-related coactivator (PRC)] related coactivator) and substrate regulation (pyruvate dehydrogenase kinase-4) increased after both E and ER, but the mRNA levels were about twofold higher after ER (P < 0.01). Phosphorylation of proteins involved in the signaling cascade of protein synthesis [mammalian target of rapamycin (mTOR), ribosomal S6 kinase 1, and eukaryotic elongation factor 2] was altered after ER but not after E. Moreover, ER induced a larger increase in mRNA of genes associated with positive mTOR signaling (ChMy and Rhei). Phosphorylation of AMP-activated protein kinase, acetyl-CoA carboxylase, and Akt increased similarly at 1 h postexercise (P < 0.01) after both types of exercise. Contrary to our hypothesis, the results demonstrate that ER, performed after E, amplifies the adaptive signaling response of mitochondrial biogenesis compared with single-mode endurance exercise. The mechanism may relate to a cross talk between signaling pathways mediated by mTOR. The results suggest that concurrent training may be beneficial for the adaptation of muscle oxidative capacity.

mitochondria; concurrent exercise; gene expression regulation; signal transduction; transcription factors/metabolism

SKELETAL MUSCLE ADAPTATION to exercise is highly dependent on the specific type of training performed. Endurance training leads to enhanced oxidative capacity and aerobic endurance, whereas heavy resistance training stimulates muscle hypertrophy and strength/power. The current paradigm is that combining endurance and strength training (concurrent training) could result in compromised muscle adaptation compared with single-mode training (4, 16, 36). Most previous studies of concurrent training have used end-point measures specific for the endurance and strength phenotypes. End-point measures of performance give limited information of the type of adaptation and of the mechanisms involved. The results from long-term training studies may also be difficult to interpret due to various confounding factors (e.g., nutrition, initial training status, and differences in trainability between subjects). Measurement of the adaptive response in molecular signaling to acute exercise may provide a deeper understanding of the mechanisms underlying training adaptation and possible interactions between signaling pathways.

The knowledge of the molecular signaling involved in the muscle adaptive response to exercise has increased considerably during the last decade. The peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) has been recognized as the main transcriptional cofactor mediating mitochondrial biogenesis and improved oxidative capacity in skeletal muscle (reviewed by Ref. 19). The PGC-1-related coactivator (PRC) belongs to the PGC-1 family and has a similar role in mitochondrial biogenesis (2). The mRNA of PGC-1α and PRC shows an early robust increase after exercise (13, 14, 25, 27, 28, 37) and can, therefore, serve as early markers of the exercise-induced adaptive response of oxidative function. The mRNA of most mitochondrial enzymes has a slower response and increases after a delay period of 10–18 h after exercise (19). Several studies have, however, shown an early increase of mRNA of pyruvate dehydrogenase kinase (PDK)4, an enzyme that regulates carbohydrate oxidation and promotes lipid oxidation (27, 37). Three kinases [p38 mitogen-activated protein kinase (MAPK), AMP-activated protein kinase (AMPK) and calcium/calmodulin-dependent protein kinase II (CaMKII)] are particularly relevant to the exercise-induced regulation of PGC-1α expression and have an important role in mediating skeletal muscle adaptations to endurance training (reviewed by Refs. 6, 19). Resistance exercise, on the other hand, is known to stimulate the mammalian target of rapamycin (mTOR) signaling pathway, which stimulates protein synthesis and muscle growth (12, 35). Recent studies have shown that mTOR can interact with the signaling cascade of mitochondrial biogenesis (11), which may provide a link between the pathways.

In vitro stimulation of rat skeletal muscle demonstrates that the activation of signaling pathways promoting endurance and strength phenotypes depend on the type of stimulation, and that interference between the pathways may occur (3). Based on these findings, it was hypothesized that the adaptations to endurance and resistance training are specific and, in part, mediated by interaction between signaling pathways (3). The findings of exercise mode-specific activation of, and/or interference between, signaling pathways (3, 4) support the idea that concurrent training may compromise the training adaptation compared with single-
mode training. Although studies in humans have shown that resistance exercise activates AMPK, the upstream regulator of mitochondrial biogenesis (10), resistance exercise had no significant effect on mRNA of PGC-1α (10) or on oxidative capacity (citrate synthase activity), as measured after 10 wk of strength training (38).

The acute molecular response to concurrent exercise was recently investigated in a model in which subjects performed resistance exercise either before or after endurance exercise (9). The results showed no increase in the phosphorylated (i.e., activated) form of AMPK and only a modest (not significant) increase in PGC-1α mRNA (9). It is not clear if the absence/minor activation of the AMPK-PGC-1α pathway after endurance exercise was due to interference from resistance exercise, or if the exercise stimulus was too low in relation to the subjects’ training status. However, since both protocols in this study (9) involved resistance exercise, it is unclear if the adaptive response to endurance exercise is affected by the addition of resistance exercise.

The aim of the present study was to investigate if resistance exercise can alter the molecular signaling response to endurance exercise in skeletal muscle. We hypothesized that the addition of resistance exercise would reduce the response to endurance exercise in the expression of marker genes related to mitochondrial biogenesis (PGC-1α, PRC) and substrate regulation (PDK4).

METHODS AND MATERIALS

Subjects. Ten healthy subjects [7 men and 3 women; age, 26 ± 1.2 (mean ± SE) yr; height, 177 ± 2.9 cm; weight, 72 ± 3.5 kg] volunteered for the present study. Subjects were recreationally active with a maximal oxygen uptake (VO2max) of 50 ± 1.9 ml·min⁻¹·kg⁻¹ and one-repetition maximum (1 RM) for leg press of 336 ± 22.3 kg, but had not been engaged in any programmed endurance or resistance exercise activity during the 6 mo before the study. The experimental procedures and potential risks associated with the study were explained to each subject, who gave written, informed consent before participation. The study was approved by the Ethics Committee of the Karolinska Institutet and performed in accordance with the principles outlined in the Declaration of Helsinki.

Experimental design. The study employed a randomized design in which each subject participated in two sessions: one with only endurance exercise (E), and the other in which endurance exercise was followed by a bout of resistance exercise (ER). The two sessions were separated by at least 2 wk for male subjects and 4 wk for female subjects (to minimize the influence of the menstrual cycle). All subjects maintained their usual dietary and physical activity patterns throughout the entire experimental period. The food intake in the evening before the first experiment was recorded and then duplicated during the evening before the second experiment. In addition, subjects were instructed to be fasted for 12 h and refrain from heavy physical exercise for at least 2 days before each trial. Subjects were fasted throughout the experiment.

Pretests. During 1-RM testing, leg press was performed with both legs at a 90–180° knee angle, and the load was progressively increased until the subjects could not perform more than one single repetition. The subjects reached 1 RM within five to six trials, and there was 4 min of rest between trials to avoid muscle fatigue. Oxygen uptake during submaximal exercise and VO2max were determined during incremental exercise to exhaustion during ergometer cycling at 60 rpm (Monark Ergomedic 893E, Monark Exercise, Varberg, Sweden). Oxygen uptake was measured using an online system (Oxycon Pro, Erich Jaeger, Hoechberg, Germany), and heart rate was recorded continuously (Polar Electro Oy, Kempele, Finland). From these measurements, a work rate corresponding to 65% of VO2max was calculated. Before exercise the cycle was adjusted (seat height and handlebar) to fit each subject, and this adjustment was maintained during the following experiments. Before the experimental sessions with muscle biopsies, subjects were familiarized with the test protocol, including both cycling and resistance exercise.

Experimental protocol. An overview of the exercise protocol is shown in Fig. 1. On the morning of the experimental day, subjects reported to the laboratory around 8 AM. After ~10-min rest in the supine position, a blood sample was taken from an arm vein, and a muscle biopsy sample was obtained from the middle portion of the vastus lateralis muscle of one leg. After 5-min warm-up on the cycle ergometer, the subjects exercised (60–70 rpm) for 60 min at a work rate corresponding to 65% of VO2max (3-min rest was allowed after 30 min of cycling; E), or performed the same cycling exercise followed by one bout of resistance exercise after 15-min rest (ER). The resistance exercise was performed in a leg press machine (243 Leg Press 45°, Gymleco, Stockholm, Sweden) and consisted of six sets of leg press at workloads corresponding to 70, 75, 80, 80, 75, and 70% of the individual 1 RM with 3-min rest between each set. During each set, subjects were encouraged to do as many repetitions as possible up to 15. One hour after the cycling exercise (i.e., 20 min following resistance exercise in the ER experiment), a second muscle biopsy and

![Test protocol A](image1)

**Test protocol A** Endurance exercise (E)

**Test protocol B** Endurance followed by resistance exercise (ER)

![Test protocol B](image2)

Fig. 1. Schematic of the experimental protocol. Endurance exercise (E) was performed as cycling at 65% of maximal oxygen uptake (VO2max), and resistance exercise (ER) as leg press at 70–80% of 1 repetition maximum with 15 repetitions or until failure. Pre, preexercise; Post, postexercise.
blood sample was taken [1 h postexercise (Post)]. A third muscle and blood sample was taken at 3 h after the cycling exercise (3 h Post) (i.e., 2 h and 20 min following resistance exercise in the ER experiment). The preexercise (Pre) biopsy was taken from one leg, and both Post biopsies (1 h and 3 h Post) were taken from the other leg. The leg chosen for the first biopsy sample was randomly assigned.

**Blood analysis.** Blood samples (4 ml) were centrifuged at 1,500 g at 4°C for 10 min. Plasma was stored at −20°C and later analyzed for lactate and glucose with enzymatic methods and for free fatty acids with a colorimetric enzymatic procedure (NEFA C test kit; Wako Chemicals, Neuss, Germany).

**Muscle biopsies.** After local anesthesia (2–3 ml Carbocain, 20 mg/ml, Astra-Zeneca, Södertälje, Sweden), an incision was made through the skin and fascia at about one-third (from patella) of the distance between the upper part of the patella and anterior superior iliac spine. A new incision was made for each biopsy. The biopsy was taken using the needle technique (7) with the application of suction. The muscle was rapidly frozen in liquid nitrogen and stored at −80°C for later analysis.

**Glycogen analysis.** Muscle biopsy samples were freeze-dried and dissected free from blood, connective tissue, and adipose tissue. Muscle glycogen content was determined according to the method previously described (22).

**Western immunoblot analyses.** Approximately 3 mg (dry wt) of dissected muscle were homogenized in a buffer (80 µl/mg) containing 20 mM HEPES, pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl2, 50 mM β-glycerophosphate, 1 mM Na3VO4, 2 mM DTT, 1% Triton X-100, 20 µg/ml leupeptin, 50 µg/ml aprotime, 40 µg/ml PMSF, and 1% phosphatase inhibitor cocktail (Sigma P-2850). Homogenates were centrifuged at 10,000 g for 10 min at 4°C to remove cell debris. The supernatant was collected, and protein concentration determination was made by BCA protein assay (Pierce Biotechnology, Rockford, IL). Aliquots of the supernatant were mixed with equal volume of Laemmli buffer and heated 5 min at 95°C to denature proteins. The gel was then incubated in transfer buffer (25 mM Tris, 192 mM glycine, and 20% methanol) at 4°C for 30 min before proteins were transferred to a polyvinylidine fluoride membrane (Bio-Rad Laboratories, Richmond, CA) for 3 h on ice in a 4°C cold room. Membranes were blocked in room temperature for 1 h in TBS (Tris-buffered saline: 10 mM Tris pH 7.6, 100 mM NaCl), containing 5% nonfat dry milk and then incubated overnight at 4°C with commercially available primary phospho-specific antibodies (Cell Signaling Technology, Danvers, MA). The primary antibodies of the phosphorylated proteins [Akt at Ser 473, mTOR at Ser 2448, ribosomal S6 kinase 1 (S6K1) at Thr 389, and eukaryotic elongation factor 2 (eEF2) at Thr 56, AMPK at Thr 172, p38 at Thr 180/Tyr182, acetyl-CoA carboxylase (ACC) at Ser 79, and CaMKII at Tyr 286] were diluted 1:1,000 in TBS with 0.1% Tween 20 (TBST) containing 2.5% nonfat dry milk. Membranes were then washed in TBST and incubated with a secondary antibody (anti-rabbit IgG conjugated with horseradish peroxidase, diluted 1:10,000; Cell Signaling Technology) during 1 h at room temperature before visualization of phosphorylated proteins by enhanced chemiluminescence. Quantification of phosphorylated proteins was then analyzed by densitometric scanning using a Gel Doc 2000 apparatus with the Quantity One version 4.6.3 software (Bio-Rad Laboratories). To verify equal loading of proteins, membranes were stained for total proteins using Memcode (Pierce Biotechnology), and the bands visually examined.

**RNA extraction and real-time PCR.** Total RNA was extracted from ~2 mg freeze-dried and dissected muscle, which was homogenized in RNA isolation reagent (PureZOL, Bio-Rad Laboratories, Sundbyberg, Sweden), according to the manufacturers’ instruction using a Polytron. The concentration as well as purity of RNA was determined by spectrophotometry, and 1 µg RNA was used to produce 20 µl of complementary DNA (cDNA) with iScript cDNA Synthesis Kit (Bio-Rad Laboratories). The primers used for the specific genes are shown in Table 1. The concentration of cDNA, annealing temperature, and PCR cycle protocol were optimized for each primer pair. The obtained cDNA was diluted ~50 times for most genes, but 400 times dilution was used for GAPDH. The amplification efficiency for all primers was 93–107%. All samples were run in duplicate, and the standard deviation of cycle threshold (Ct) values of each duplicate was <0.3%. Samples from one subject were run on the same plate to permit direct relative comparisons. The specificity of the PCR reaction was evaluated by melting curve analysis after the final PCR cycle. A single peak was observed for each sample, which demonstrates the absence of primer dimers and other nonspecific product amplification.

Real-time qPCR amplification mixtures (25 µl) contained 12.5 µl 2× SYBR Green Supermix (Bio-Rad Laboratories), 0.2 µM of the forward and reverse primers, and 11.5 µl template cDNA diluted in RNase-free water. Real-time PCR was performed with Bio-Rad iCycler (Bio-Rad Laboratories). Relative changes in mRNA levels were analyzed by 2^ΔΔCt, and GAPDH was used as the reference gene. Ct values for GAPDH remained unchanged during both E (Pre: 18.5 ± 0.2; 1 h Post: 18.6 ± 0.4; 3 h Post: 18.2 ± 0.2) and ER (Pre: 18.3 ± 0.3; 1 h Post: 18.9 ± 0.6; 3 h Post: 18.5 ± 0.3).

**Statistical analyses.** All data are expressed as means ± SE and analyzed by two-way repeated-measures ANOVA (time and exercise model). When a significant main effect was observed post hoc analyses (Fisher least significant difference) was performed to locate the difference. For some positively skewed distributed variables, log-transformation was performed before statistical analyses. Statistical significance was accepted at P < 0.05.

## RESULTS

**Physiological and metabolic responses to exercise.** All subjects completed two sessions of 60-min cycling with an identical work rate during both sessions (161 ± 12 W corresponding to 65 ± 1.4% of VO2max). The load during leg press ranged from 70% to 80% of 1 RM, and each set was performed until failure or until 15 repetitions (Table 2). The average work performed during resistance exercise (vertical distance × load × repetitions) was 33 kJ, which is ~6% of that during the 60-min cycling session. Plasma glucose concentration decreased over time, and plasma free fatty acid was increased threefold without difference between exercise modes (Table 3). Plasma lactate concentration increased in ER (1 h Post, but was unchanged in E (Table 3). Muscle glycogen content was reduced to 42% (E) and 34% (ER) of the initial level at 1 h Post and remained reduced at 3 h Post. There was no significant difference between exercise modes in muscle glycogen depletion (Table 2).

**Induction of genes related to oxidative metabolism.** See Fig. 2. The mRNA of key marker genes of mitochondrial biogenesis (PGC-1α, PRC) and substrate regulation (PDK4) increased significantly after both models of exercise. In E (3 h Post), the mRNA of PGC-1α, PRC, and PDK4 was increased 10-, 2-, and 14-fold, respectively. The adaptive response was, however, more pronounced after ER, and at 3 h Post mRNA was about twofold higher after ER than after E (PGC-1α 1.9-fold higher, P < 0.05; PRC 1.7-fold higher, P < 0.01; PDK4 2.2-fold higher, P < 0.01). The mRNA content of peroxisome proliferator-activated receptor-β/δ, ALAS1, and succinate dehydrogenase (Table 4, Fig. 2) did not change in any of the situations, whereas SIRT1 was reduced 1 and 3 h Post in both E and ER (Table 4).
Phosphorylation of proteins. See Figs. 3 and 4. There was a significant difference between ER and E in the exercise-induced phosphorylation of key enzymes in protein synthesis i.e., mTOR, mammalian target of rapamycin; PDK4, pyruvate dehydrogenase kinase, isozyme 4; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PPAR-β/δ, peroxisome proliferator-activated receptors β/δ; PRC, PGC-1-related coactivator; REDD1/2, regulated in development and DNA damage response 1/2; Rheb, ras-homolog enriched in brain; S6K1, p70 ribosomal protein S6 kinase 1; SDH, succinate dehydrogenase; SIRT1, sirtuin (silent mating type information regulation 2 homolog); TSC1/2, tuberous sclerosis.

<table>
<thead>
<tr>
<th>Genes</th>
<th>General Description of Protein</th>
<th>Forward</th>
<th>Reverse</th>
<th>No. in Genbank</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAS1</td>
<td>Nuclear-encoded mitochondrial enzyme</td>
<td>GGCACGACCAGATGAACTGAGAG</td>
<td>TCTAGAACCCTTCTTCGCAG</td>
<td>NM_199166</td>
</tr>
<tr>
<td>cMyc</td>
<td>Downstream target of mTOR; positive regulator of ribosomal biogenesis</td>
<td>CGTCTGCCACAGCTAGGACCAA</td>
<td>TCTGGGACACGATAGTCCTT</td>
<td>NM_002467</td>
</tr>
<tr>
<td>hVps34</td>
<td>Nutrient regulator of mTOR</td>
<td>GAGCAAGATGGAATGAACCC</td>
<td>CCAGGACATCTACTTGACC</td>
<td>NM_002467</td>
</tr>
<tr>
<td>mTOR</td>
<td>Nutrient regulator of protein synthesis</td>
<td>CTCGGCTTGTTGAGTTGTCC</td>
<td>GATTTACGCGCCTTCCTTGG</td>
<td>NM_004985</td>
</tr>
<tr>
<td>PDK4</td>
<td>Inhibits CHO oxidation and promotes lipid oxidation</td>
<td>TCCAGCTGAGCCACAGGCT</td>
<td>TGGAGAACGCTTAGACAAA</td>
<td>NM_002612</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Transcriptional coactivator involved in mitochondrial biogenesis</td>
<td>TTCTTTTGCGACATGAACTC</td>
<td>AAGACAGAAGTGAACAGC</td>
<td>AF106998</td>
</tr>
<tr>
<td>PPAR-β/δ</td>
<td>Transcriptional factor involved in metabolism</td>
<td>ATGGAGACCCAGAACAGGAGACG</td>
<td>GCATGAGGCCCCGTCAAGC</td>
<td>NM_006238</td>
</tr>
<tr>
<td>PRC</td>
<td>Transcriptional coactivator involved in mitochondrial biogenesis</td>
<td>GCCTGCAAAAGGATTCTCCCG</td>
<td>AAAGTCTTTCCGTTGAGT</td>
<td>AF325193</td>
</tr>
<tr>
<td>REDD1</td>
<td>Negative regulator of mTOR</td>
<td>CTGGAGGACCTGTCGTGCA</td>
<td>TCCGAGTTAGGGCCTTGGTC</td>
<td>NM_001905</td>
</tr>
<tr>
<td>REDD2</td>
<td>Negative regulator of mTOR</td>
<td>CCCAAGAGAGCTTGCTAAAGTG</td>
<td>TTTGCTTTGATGTTGACAGCA</td>
<td>NM_145244</td>
</tr>
<tr>
<td>Rheb</td>
<td>Positive regulator of mTOR</td>
<td>TTTTTGGGACTTTGCTGTAAGAAA</td>
<td>AAGACTTCGGTTGAGAAGCTG</td>
<td>NM_005614</td>
</tr>
<tr>
<td>S6K1</td>
<td>Downstream target of mTOR; positive regulator of protein synthesis</td>
<td>ACTCAATTTGTCCTCCCTAC</td>
<td>AGAATGAGTGAAGTTGACCTT</td>
<td>NM_003161</td>
</tr>
<tr>
<td>SDH</td>
<td>Mitochondrion enzyme</td>
<td>TACGGTTGACCGAGAAGTTGACAGCC</td>
<td>GCCTGCTTCTAGGCTGAGATGCTG</td>
<td>DI0245</td>
</tr>
<tr>
<td>SIRT 1</td>
<td>NAD-dependent deacetylase that promotes activation of PGC-1α</td>
<td>TACCGAGAAGACAGAGGAAAGC</td>
<td>AAGGTTATCTGGTATACCAAATCG</td>
<td>NM_012238</td>
</tr>
<tr>
<td>TSC1</td>
<td>Negative regulator of mTOR</td>
<td>TAAAGCTGAGGAGCTGTTGAGG</td>
<td>CTCGTTGCTTTGAGTCAG</td>
<td>NM_000568</td>
</tr>
<tr>
<td>TSC2</td>
<td>Negative regulator of mTOR</td>
<td>CCCGAGACATGAGTGGCT</td>
<td>CACTGAGTGGAGGAGCTGCG</td>
<td>NM_000548</td>
</tr>
</tbody>
</table>

Table 1. Details of primers used for RT-qPCR

Table 2. Details of the performed resistance exercise

<table>
<thead>
<tr>
<th>Resistance Exercise</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
<th>Set 4</th>
<th>Set 5</th>
<th>Set 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>235 ± 15</td>
<td>249 ± 16</td>
<td>264 ± 17</td>
<td>264 ± 17</td>
<td>249 ± 16</td>
<td>235 ± 15</td>
</tr>
<tr>
<td>% of 1 RM</td>
<td>70.2 ± 0.5</td>
<td>74.8 ± 0.8</td>
<td>78.7 ± 0.8</td>
<td>78.7 ± 0.8</td>
<td>74.4 ± 0.8</td>
<td>70.2 ± 0.8</td>
</tr>
<tr>
<td>Repetition, times</td>
<td>14.4 ± 1.0</td>
<td>11.1 ± 0.6</td>
<td>9.5 ± 0.7</td>
<td>8.7 ± 0.8</td>
<td>10.1 ± 0.9</td>
<td>10.7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE from 10 subjects. Resistance exercise was performed as leg press. Each set was performed until failure or with 15 repetitions. 1 RM, one repetition maximum.
Table 3. Plasma metabolites and muscle glycogen content

<table>
<thead>
<tr>
<th>Type</th>
<th>Pre</th>
<th>1 h Post</th>
<th>3 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA, mmol/l</td>
<td>E</td>
<td>0.07 ± 0.01</td>
<td>0.21 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>0.07 ± 0.03</td>
<td>0.20 ± 0.04*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>E</td>
<td>5.18 ± 0.07</td>
<td>5.02 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>5.42 ± 0.21</td>
<td>5.24 ± 0.14</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>E</td>
<td>1.14 ± 0.17</td>
<td>1.15 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>1.20 ± 0.18</td>
<td>6.64 ± 0.83*</td>
</tr>
<tr>
<td>Glycogen, mmol/kg dry wt</td>
<td>E</td>
<td>460 ± 21</td>
<td>194 ± 35*</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>434 ± 26</td>
<td>146 ± 20*</td>
</tr>
</tbody>
</table>

Values are means ± SE from 10 subjects. Due to limited amount of material, muscle glycogen could only be measured in 7 subjects. FFA, free fatty acid; E, endurance exercise; ER, endurance exercise followed by resistance exercise; Pre, preexercise; Post, postexercise. *P < 0.01 vs. Pre. 

Induction of genes related to mTOR signaling. See Fig. 5. There were significant increases in mRNA of proteins related to activation of mTOR signaling (Rheb, cMyc) at 1 and 3 h Post in both exercise models (P < 0.01), but the adaptive response of Rheb and cMyc was more pronounced (P < 0.05 or < 0.01) after ER than after E. The mRNA of mTOR, S6K1, and the nutrient regulator of mTOR (hVps34) did not change significantly after exercise in any situation (Table 4). The induction of genes related to negative mTOR signaling showed a heterogeneous response after exercise. The mRNA of regulated in development and DNA damage response 1 (REDD1) increased 1 h Post (P < 0.01) but declined below the Pre level 3 h Post, without difference between exercise models. The mRNA of REDD2 was reduced 3 h Post (P < 0.01) without difference between exercise models. Thiazide-sensitive Na-Cl cotransporter (TSC) 2 was elevated 10.2 ± 2.47 on April 5, 2017 http://jap.physiology.org/ Downloaded from 1.5-fold 1 h Post in both E and ER, but TSC1 did not change significantly in any of the situations (Table 4).

Fig. 2. Abundance of mRNA of genes associated with oxidative metabolism and mitochondrial biogenesis. Muscle samples were taken Pre, 1 h Post, and 3 h Post. Values of the target genes are expressed in relation to the reference gene (GAPDH). Values are means ± SE for 10 subjects (n = 9 for SDH). A: PGC-1α. B: PRC. C: PDK4. D: SDH. For definition of genes, see Table 1 legend. *P < 0.05 and **P < 0.01 vs. Pre. #P < 0.05 and ##P < 0.01, ER vs. E.
DISCUSSION

This is the first study to investigate if heavy resistance exercise alters the adaptive response of molecular signaling to endurance exercise. A remarkable novel finding in the present study is that resistance exercise performed after endurance exercise results in enhanced expression of genes involved in the signaling cascade of mitochondrial biogenesis/oxidative metabolism, as well as enhanced activation of proteins involved in the regulation of protein synthesis.

The mRNA of key proteins involved in the regulation of mitochondrial biogenesis (PGC-1α, PRC) or substrate regulation (PDK4) was about twofold higher when resistance exercise was added to endurance exercise compared with single-mode endurance exercise. The gene expression of PGC-1α is induced by increased activation (i.e., phosphorylation) of the energy-sensing proteins AMPK and p38 MAPK (20, 29), as well as by Ca2+-induced activation of CaMKII (19, 21). It has recently been reported that high-intensity exercise results in higher PGC-1α mRNA abundance than isocaloric low-intensity exercise, and that this was associated with enhanced AMPK signaling, but without differences in p38 MAPK activation (13). In the present study, phosphorylation of AMPK, as well as one of its targets (ACC), increased similarly in both protocols (1 h Post). The enhanced PGC-1α mRNA abundance following concurrent exercise can, therefore, not be explained by increased AMPK activation. However, AMPK activation is most marked during/immediately after exercise, and it cannot be excluded that the timing of biopsies was too late to detect differences in AMPK activation.

Our results on p38 MAPK showed a tendency of increased phosphorylation in ER at 1 h Post, but the results show great variability. The Ca2+-sensitive protein CaMKII was not activated after exercise, but it cannot be excluded that the timing of muscle biopsies may be inappropriate to detect early Post changes in CaMKII phosphorylation. Although p38 MAPK, AMPK, and CaMKII have been recognized as important upstream proteins linked to PGC-1α signaling (19, 20, 29), other factors, such as reactive oxygen species (32) and blood lactate...
have also been implicated as regulators of PGC-1α gene expression. Hence, it is possible that increased levels of reactive oxygen species or lactate concentration in the combined protocol may have contributed to enhanced expression of PGC-1α after ER. Furthermore, there is evidence that the activity of mTOR may have a critical role in modifying oxidative function and mitochondrial biogenesis (11, 31, 39). Pharmacological inhibition of mTOR with rapamycin resulted in reduced gene expression of PGC-1α in cells and mouse skeletal muscle (11), as well as reduced mitochondrial activity and capacity (31, 39). The interaction seems to be mediated through the transcription factor yin-yang 1, which has been identified as a common target of mTOR and PGC-1α (11). The present study demonstrates that ER results in a robust increase in the phosphorylation/activation of mTOR, whereas p-mTOR remained unchanged after E. The activation of mTOR after ER, but not after E, may thus explain the enhanced gene expression of PGC-1α after ER. An interaction between mTOR and PGC-1α would suggest that a cross talk exists between the signaling pathways, promoting strength and endurance phenotypes. In contrast to the AMPK-Akt interaction (3), which is inhibitory, the mTOR-PGC-1α interaction could instead stimulate mitochondrial biogenesis.

The experimental protocol in this study was designed to investigate the hypothesis that resistance exercise reduces the signaling response to endurance exercise. This mimics a “real-life” situation, where subjects, after having terminated a session of endurance exercise, continue with a session of resistance exercise. In contrast to our hypothesis, we found increased mRNA of marker genes of mitochondrial biogenesis, and it is evident that resistance exercise does not impair the signaling response after endurance exercise, as previously suggested (4, 16). We did not determine the effect of single-mode resistance exercise in this study, and it is, therefore, not known if the enhanced expression of genes related to mitochondrial biogenesis after ER is due to an effect of the additional exercise or to a synergistic effect. The amount of performed work during ER was only 6% higher than during E and corresponds to 4-min cycling. It is highly unlikely that an additional 4 min of cycling in E should result in such a dramatic boosting effect of the oxidative gene transduction as that observed after ER. However, the stimulus for gene trans-
duction might be more dependent on the intensity of the exercise than the actual amount of work performed (24). Recent studies demonstrate that short-term intermittent high-intensity cycling can increase mRNA of PGC-1α in both moderately trained (14) and endurance-trained subjects (28). We cannot exclude that combining traditional endurance exercise with high-intensity sprint exercise would give a similar boosting effect as that observed after ER.

Post biopsies in the present study were taken 1 and 3 h after termination of endurance exercise, which means that biopsies in ER were taken 0.33 and 2.33 h after termination of resistance exercise. The choice of this experimental model was based on the knowledge that endurance exercise is the primary stimulus of mitochondrial biogenesis, and that ethical considerations prevented further muscle biopsies. The increase in PGC-1α mRNA is delayed after exercise, and the peak value is normally reached after 2–5 h of recovery (25, 27) and is thereafter maintained at the peak value for several hours (25). Our results may, therefore, if anything, underestimate the difference between ER and E in mRNA of PGC-1α at 1 h Post, whereas the difference between 2.33 and 3 h is likely minimal.

Another interesting finding was that endurance exercise had only a small influence on the signaling pathway promoting protein synthesis (no change in p-mTOR). This finding is consistent with that after endurance-type stimulation in rat muscle (3), but different from one-legged endurance exercise in humans (38). ER resulted in enhanced phosphorylation of mTOR and its downstream target S6K1, as well as larger activation of eEF2 (decreased phosphorylation) compared with single-mode E. Our results are consistent with the current view that resistance exercise promotes the signaling pathway of protein synthesis, whereas single-mode endurance exercise has much less effect on this pathway. Although ER enhanced the adaptive response in the phosphorylation of mTOR and its downstream targets S6K1 and eEF2, there was no difference between exercise models in the phosphorylation of the upstream regulator Akt, suggesting that this is not the sole mechanism in mTOR activation. With the reservation that the timing of the biopsies is different in E and ER, these observations suggest that mTOR and downstream signaling are influenced by additional inputs, such as phospholipase D1 and phosphatidic acid, which seems to be involved in mediating exercise signaling via mechanotransduction (18, 34).

The gene expression level of cMyc and Rheb (proteins related to activation of mTOR) increased to a larger extent by ER, whereas gene expression of REDD1, REDD2, and TSC2 (proteins related to inactivation of mTOR) showed similar changes in mRNA content following the two protocols. The changes in gene expression of proteins regulating mTOR may be important for the adaptive response during long-term training, but cannot explain the observed acute changes in the activation of mTOR and its downstream targets. Hence, the increase in Rheb and cMyc mRNA, an effect that was enhanced by resistance exercise, in concert with the decrease in

Fig. 5. Abundance of mRNA of genes associated with mTOR regulation. Rheb and cMyc are positive, whereas REDD1 and REDD2 are negative modulators of mTOR activity. Muscle samples were taken Pre, 1 h Post, and 3 h Post. Values of the target genes are expressed in relation to the reference gene (GAPDH). Values are presented as means ± SE for 10 subjects. For definition of genes, see Table 1 legend. *P < 0.05 and **P < 0.01 vs. Pre, #P < 0.05 and ##P < 0.01, ER vs. E.
REDD1/2 mRNA content at 3 h Post may all be related to a long-term enhancement of mTOR signaling and synthesis of new ribosomes (40).

Some (17, 26, 33), but not all (5, 8, 23), studies have shown that concurrent training of endurance trained subjects results in improved endurance compared with single-mode endurance training. The improved endurance after concurrent training might be explained by increased economy of movement (possibly related to increased strength and altered neuromuscular function) (1), but not by increased VO2max, which remained unchanged (17, 26, 33). Only a few studies have investigated the effect of concurrent training on muscle oxidative enzymes (i.e., markers of mitochondrial density). Adding resistance exercise to an endurance training program had no effect on the activity of citrate synthase when the sessions were separated by >1 h (8, 17, 30). However, when resistance and endurance exercise were combined into one session, citrate synthase activity increased 26% after 20 wk of training (30). These findings suggest that the timing of resistance and endurance exercise is important for the muscle adaptive response. In most studies of concurrent training, the bouts of endurance and resistance exercise have been performed on different days or separated by several hours, whereas, in the present study, resistance exercise was performed 15 min after the endurance exercise. Close vicinity between endurance and resistance exercise may be critical for obtaining an enhanced signaling response.

In summary, the present study shows that combining endurance and resistance exercise leads to larger increases in the mRNA of early markers of mitochondrial biogenesis (PGC-1α and PRC) and substrate regulation (PDK4) compared with single-mode endurance exercise. The combined exercise protocol also resulted in a robust phosphorylation/activation of mTOR and its downstream target S6K1, whereas single-mode endurance exercise had no influence on this signaling pathway. The enhanced signaling response of mitochondrial biogenesis after ER may be related to a cross talk between signaling pathways mediated through the activity of mTOR, which, in addition to muscle growth, also regulates the expression of mitochondrial genes (11). The results from this study challenge the current view of how to optimize endurance training and indicate that a bout of resistance exercise performed shortly after endurance exercise can improve the training-induced stimulation of mitochondrial biogenesis. The exercise-induced increase in molecular signaling is the first crucial step in the adaptive response and may lead to subsequent changes in protein abundance. However, it is clear that the outcome of training is influenced by a number of additional processes/factors, such as the translation process and the balance between synthesis and degradation of proteins. Further studies are required to investigate if long-term training with the currently used protocol is an effective strategy to improve muscle oxidative capacity.

ACKNOWLEDGMENTS

We thank all the participants for precious time and effort. We also gratefully acknowledge Marjan Pontén for excellent technical assistance.

GRANTS

The study was supported by grants from the Swedish National Centre for Research in Sports, the Swedish Research Council, and the Swedish School of Sport and Health Sciences, Stockholm, Sweden.

DISCLOSURES

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

REFERENCES

22. Leighton B, Blomstrand E, Challiss RA, Lozman FJ, Parry-Billings M, Dimitriadis GD, Newsholme EA. Acute and chronic effects of...


