INFLUENCE OF RESISTANCE EXERCISE INTENSITY AND METABOLIC STRESS ON ANABOLIC SIGNALING AND EXPRESSION OF MYOGENIC GENES IN SKELETAL MUSCLE

DANIIL V. POPOV, PhD,[1] EVGENY A. LYSENKO, PhD,[1] ANTON V. BACHININ,[1] TATIANA F. MILLER, PhD,[1] NADEZDA S. KUROCHKINA,[2] IRINA V. KRAVCHENKO, PhD,[3] VLADIMIR A. FURALYOV, PhD,[3] and OLGA L. VINOGRADOVA, PhD[1]

[1] Laboratory of Exercise Physiology, Institute of Biomedical Problems, Russian Academy of Sciences, 76A Khoroshevskoe Shosse, Moscow 123007, Russia

[2] Faculty of Fundamental Medicine, M.V. Lomonosov Moscow State University, Moscow, Russia

[3] Laboratory of Enzyme Engineering, A.N. Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia

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ABSTRACT: *Introduction:* We investigated the effect of resistance exercise intensity and exercise-induced metabolic stress on the activation of anabolic signaling and expression of myogenic genes in skeletal muscle. *Methods:* Ten strength-trained athletes performed high-intensity [HI, 74% of 1-repetition maximum (RM)], middle-intensity (MI, 54% 1RM), or middle-intensity (54% 1RM) no-relaxation exercise (MIR). Kinase phosphorylation level and myogenic gene expression in muscle samples were evaluated before, 45 min, 5 h, and 20 h after exercise. *Results:* The lactate concentration in MI was approximately 2-fold lower than in the 2 other sessions, and was highest in MIR. The phosphorylation level of extracellular kinase $1/2^{Thr202/Tyr204}$ after exercise was related to metabolic stress. Metabolic stress induced a decrease in myostatin mRNA expression, whereas mechano-growth factor mRNA level depended on exercise intensity. *Conclusions:* This study demonstrates that both intensity and exercise-induced metabolic stress can be manipulated to affect muscle anabolic signaling.

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An increase in muscle mass and strength is an important goal in rehabilitation medicine, sarcopenia, and protection from inactivity in elderly subjects and for athletic training. According to the recommendations of Spiering *et al.*,[1] the intensity of resistance exercise should be >70% of 1-repetition maximum (1RM). It is known that the mechanical load affects the extracellular matrix of muscle fibers and regulates muscle mass via integrin-associated focal adhesion kinase (FAK),[2] generation of phosphatidic acid,[3] activation of mitogen-activated protein kinases (MAPK),[4] and expression of mechano-growth factor (MGF or insulinlike growth factor-1Ec).[5] Several studies have investigated the effect of resistance exercise intensity on anabolic signaling, regulation of myogenic gene expression, and myofibrillar protein synthesis rate after acute exercise sessions.[6–9] Interestingly, it was impossible to unambiguously evaluate the effects of resistance exercise intensity, because the training loads in those studies differed in intensity, total number of contractions, and total tension time.

High-intensity resistance exercise may be excessively strenuous, especially for the elderly, and it may lead to exercise-induced damage. Therefore, the anabolic potential of low- and middle-intensity resistance exercise (20−50% of 1RM) with blood flow restriction in working muscles has been investigated intensively during the last decade. Blood flow restriction during an exercise bout may be achieved by vascular occlusion[10] or with a special type of "no-relaxation" exercise of trained muscles.[11–13] The essence of no-relaxation exercise is that, during rhythmic extension−flexion movements, the muscles do not relax at the end of each movement cycle (i.e., the next extension is started immediately after flexion). Previous studies have shown that several weeks of low- or medium-intensity resistance training (20−50% of 1RM) with blood flow restriction led to a greater increase in muscle mass and strength compared with traditional training at the same exercise intensity.[10,14]

Skeletal muscle hypertrophy induced by low-intensity resistance exercise with blood flow restriction is associated with substantial intramuscular metabolic stress, which stimulates growth hormone and insulin-like growth factor-1 (IGF-1) secretion[11,15] and activates mammalian target of rapamycin complex 1 (mTORC1), extracellular kinase 1/2 (ERK1/2) signaling,[16,17] and expression of myogenic regulatory genes.[18] The mechanisms underlying muscle

Abbreviations: 1RM, 1-repetition maximum; AMPK, 5′-AMP-activated protein kinase; ATP, adenosine triphosphate; ERK1/2, extracellular kinase 1/2; FAK, focal adhesion kinase; FOXO, forkhead box protein; HI, high-intensity resistance exercise session; IGF-1, insulin-like growth factor 1; MAPK, mitogen-activated kinase; HIF-1, hypoxia-inducible factor 1; MGF (IGF-1Ec) mechano-growth factor (insulin-like growth factor 1, isoform 1Ec); MI, middle-intensity resistance exercise session; MIR, middle-intensity no-relaxation resistance exercise session; mTORC1, mammalian target of rapamycin complex 1; MF, muscle fiber; MyoD1, myogenic differentiation 1; p21, cyclin-dependent kinase inhibitor 1A; p70S6K, p70 ribosomal S6 kinase; p90S6K, p90 ribosomal S6 kinase; PCR, polymerase chain reaction; ROS, reactive oxygen species; SSC, stretch−shortening cycle.

Key words: gene expression; metabolic stress; protein kinase; resistance exercise; skeletal muscle

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Correspondence to: D.V. Popov; e-mail: danil-popov@yandex.ru

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hypertrophy induced by resistance exercise with blood flow restriction have been investigated only for low-intensity exercise (20% of 1RM). Notably, these studies compared low-intensity exercise with blood flow restriction with traditional resistance exercise of equal intensity (20% of 1RM) and work. It is well known that traditional low-intensity resistance exercise performed without substantial fatigue does not increase muscle protein synthesis,[6,17] growth of muscle mass, or strength.[14]

Suga *et al.* used ^{31}P-spectroscopy to demonstrate that, to achieve intramuscular metabolic stress comparable to high-intensity resistance exercise (65% of 1RM), the exercise intensity with blood flow restriction should be >30% of 1RM.[19] Moreover, a middle-intensity (50% of 1RM) no-relaxation exercise session induced more lactate accumulation in the blood compared with a traditional high-intensity (80% of 1RM) session, and several weeks of middle-intensity training led to an increase in muscle mass and strength that was comparable to that of high-intensity training.[11] To date, the molecular mechanisms of adaptation to middle-intensity exercise with blood flow restriction remain unclear.

The goal of this study was to evaluate the effects of resistance exercise intensity and metabolic stress on the activation of intramuscular anabolic signaling and on the expression of myogenic regulatory genes. We investigated the effects of a high-intensity (75% of 1RM) resistance exercise session (HI), a middle-intensity (50% of 1RM) exercise (MI) session, and a middle-intensity (50% of 1RM) no-relaxation exercise session (MIR), which had equivalent total tension time, total number of contractions, and range of motion. We used the exercise of small muscle mass (bilateral leg extension−flexion), because the secretion of anabolic hormones depends on the muscle mass involved.[20,21] This approach allowed us to avoid exercise-induced blood hormone increases that could influence anabolic signalling, specifically insulin and IGF-1 affecting Akt-mTORC1 and testosterone influencing protein synthesis rate.[22] We hypothesized that increasing the exercise intensity from 50% of 1RM (MI) to 75% of 1RM (HI) would induce activation of intramuscular anabolic signaling and expression of myogenic regulatory genes. In addition, we hypothesized that MIR, even for middle-intensity exercise, would activate the mechanisms that underlie muscle protein synthesis and myogenesis by producing strong metabolic stress.

METHODS

Ethics Approval. This study was approved by the human ethics committee of the Institute of Biomedical Problems. All participants provided written consent to take part in the study. The investigation complied with the guidelines set forth in the Declaration of Helsinki.

Initial Study. Ten amateur athletes (sprinters and middle-distance runners) and physically active men with a median weight of 76 (interquartile range 72–80) kg, height 1.78 (1.74–1.88) m, and age 23.1 (21.7–24.6) years participated in this study. All participants usually perform 1 or 2 strength training sessions per week. During the first 2 weeks of the study (4 visits to the laboratory), the subjects were familiarized with the 1RM test and exercise protocols. For simultaneous loading of both legs during exercise (bilateral knee extension) 2 dynamometers (Pro System 3; Biodex, USA) were mounted on 1 bed. The levers of the dynamometers were secured, and equal ranges of motion were set for both. The torque and angle analog signals were converted by an analog-to-digital converter (Model E-440; L-card, Russia) and recorded by PowerGraph 3.3 software (DISoft, Russia). The signals from both dynamometers were averaged and displayed online. After warm-up 1RM was determined; each participant performed a single bilateral knee extension in isotonic mode with a torque of 140 N•m. After a 1-min rest, the torque was increased by 10 N•m. The 1RM was determined as the maximal torque when a subject could turn the dynamometer levers from the initial position (knee joint angle 90°) to a 50° angle.

Primary Study. All subjects performed 3 separate resistance test sessions, each once per week in a randomized order. All participants were instructed to refrain from resistance exercise for 1 week and to refrain from all exercise 36 h before the test. The test session consisted of a warm-up (10 min of cycling, workload 1 W/kg of body weight) and 8 sets of 12 bilateral knee extensions−flexions separated by 6-min rest periods. The dynamometer torque levels were set at 50% of 1RM for MI and MIR and at 75% of 1RM for HI. The knee extensions and flexions were performed in isotonic concentric and eccentric modes, respectively. The rhythm of the movements was set by visual and sound signals using custom software. The extension and flexion times were 0.4 s and 1.9 s, respectively. The total tension time was equal in all training sessions (Fig. 1). During MI and HI, the rest period between extension−flexion cycles was 3 s. During MIR, each extension was started immediately after a flexion. Therefore, the knee extensor muscles continuously produced tension during a set so that the torque during a set was maintained at an approximately constant level (Fig. 1). The knee angle and the real torque produced by the knee extensors during exercise were recorded as described above.

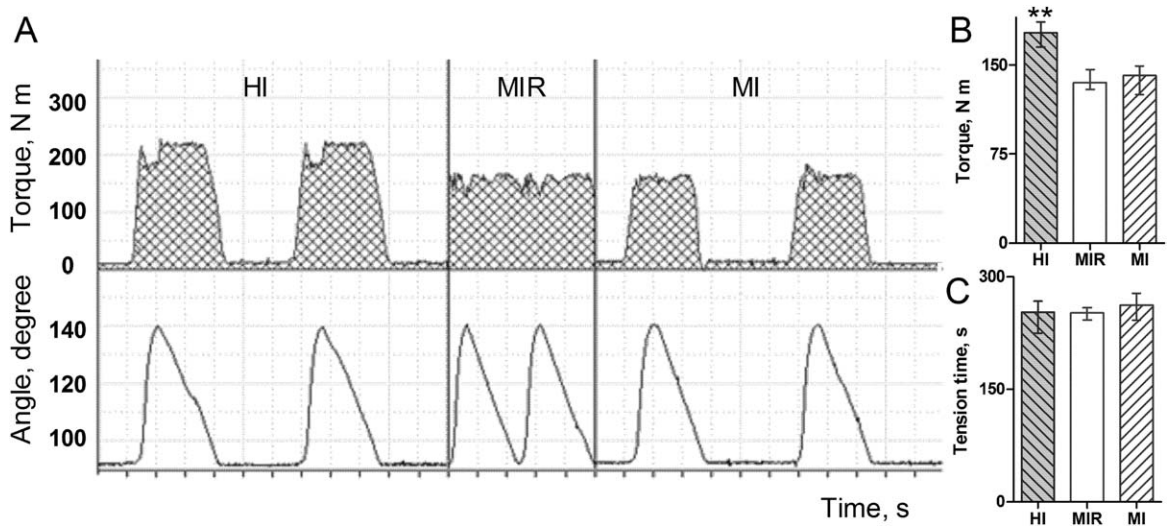

FIGURE 1. (A) Representative dynamics of torque and the knee angle during high-intensity resistance exercise (HI), middle-intensity resistance exercise (MI), and middle-intensity resistance no-relaxation exercise (MIR). During MIR, the next knee extension was started immediately after flexion so the torque and the muscle tension were practically constant, which produced a restriction of blood flow in the working muscles. **(B)** Average torque during the exercise sessions. **(C)** Average tension time during the exercise sessions. Values are expressed as median and interquartile range. $^{**}P < 0.01$ difference vs. MIR and MI.

The participants arrived at the laboratory at 9:40 A.M. and consumed a standard breakfast (3624 kJ; 24 g of protein, 157 g of carbohydrates, and 15 g of lipids). The exercise session started 1 h 40 min after breakfast. All participants consumed 24 g of carbohydrates, 5 g of branched-chain amino acids (2.5 g of leucine, 1.25 g of isoleucine, and 1.25 g of valine), and *ad libitum* water during the second part of the exercise session and also consumed a standard lunch (3650 kJ; 29 g of protein, 116 g of carbohydrates, and 43 g of lipids) 60 min after termination of the exercise. All participants had their usual dinner at home prior to 9:00 P.M. and returned to the laboratory the next morning at 8:00 A.M. in the fasted state for muscle biopsy.

Venous blood was drawn before and 15 min after exercise for evaluation of testosterone and insulin using an immunoassay system (DxI 800; Beckman Coulter, UK) and of IGF-1 using another immunoassay system (Immulite 1000; Siemens, Germany). Blood was drawn from fingertip capillaries 30 s after 3, 5, and 8 sets for determination of lactate and glucose concentrations (Super GL Easy Analyzer; Dr. Mueller Geraetebau GmbH, Germany). Biopsies were taken from the vastus lateralis muscle using the microbiopsy technique[23] before and at 45-min, 5-h, and 22-h intervals after exercise under local anesthesia (2 ml of 2% lidocaine). The muscle samples were quickly blotted with gauze to remove superficial blood, frozen in liquid nitrogen for 20 s, and stored at −80°C until analysis. The first biopsy was taken 12 cm proximal to the lateral femoral condyle. Subsequent biopsies were taken 2 cm proximal to the previous biopsy. The biopsies were taken from the right leg during the first and third test sessions and from the left leg during the second test session.

Immunoblotting. Frozen samples (~10 mg) were sectioned at 20 μm by an ultratome (Leica, Germany) and homogenized in ice-cold radioimmunoprecipitation 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 phenylmethylsulfonylfluoride). Samples were then centrifuged for 10 min at 10,000 × *g* and 4°C. Protein content was analyzed by the bicinchoninic assay. The samples (20 μg protein per lane) were mixed with Laemmli buffer and loaded onto a 10% T polyacrylamide gel. Electrophoresis was performed using the Mini-Protean Tetra Cell system (Bio-Rad, USA) at 20 mA per gel. The proteins were transferred onto a 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, which was followed by washing and incubation in 5% nonfat dry milk for 1 h. The membrane was then incubated at 25°C with anti−phospho-p70S6K^{Thr389} (Santa Cruz Biotechnology, Germany) for 2 h or at 4°C with anti-p70S6K, anti-phospho-Akt^{Thr308}, anti-Akt1, anti-phospho-Erk1/2$^{Thr202/Tyr204}$, anti-Erk1/2 (all from Cell Signaling Technology, USA), anti−phospho-AMPKα1/2^{Thr172}, and anti-AMPKα1/2 (Santa Cruz Biotechnology, Germany) overnight. On the next day, the membrane was incubated with anti-rabbit secondary antibody (Cell Signaling, USA) for 1 h. After each

Table 1. Primer sequences.

Gene	Forward (5′–3′)	Reverse (5′–3′)
p21	CCTCATCCCGTGTTCTCCTTT	GTACCACCCAGCGGACAAGT
MyoD1	GGTCCCTCGCGCCCAAAAGAT	CAGTTCTCCCGCCTCTCCTAC
IGF-1Ea	ATGCTCTTCAGTTCGTGTGTG	GCACTCCCTCTACTTGCGTTC
MGF (IGF-1Ec)	ACCAACAAGAACACGAAGTC	CAAGGTGCAAATCACTCCTA
Myostatin	CATGATCTTGCTGTAACCTTCC	CGATAATCCAATCCCATCC
RPLP0	CACTGAGATCAGGGACATGTTG	CTTCACATGGGGCAATGG
ACTB	CGTGACATTAAGGAGAAGCTGTGC	CTCAGGAGGAGCAATGATCTTGAT

p21, cyclin-dependent kinase inhibitor 1A; MyoD1, myogenic differentiation 1; IGF-1Ea, insulin-like growth factor-1, splice variant Ea; MGF or IGF-1Ec, insulin-like growth factor-1, splice variant Ec; RPLP0, ribosomal protein, large, P0; ACTB, actin, beta.

step, the membrane was washed with PBS-Tween 20 (3 washes for 5 min each). The membrane was incubated with enhanced chemiluminescence 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). All data are expressed as the ratio of phosphorylated to total protein.

RNA Extraction. RNA was extracted from approximately 20 mg of wet muscle using TRIzol (Invitrogen, USA). RNA concentration was measured by spectrophotometry (BioPhotometer, Eppendorf, Germany) at an absorbance of 260 nm, and RNA purity was assessed by the A260/A280-nm absorption ratio. cDNAs were obtained by annealing 1.5 μg of denatured (70°C for 5 min) total RNA with oligo $(dT)_{15}$ at 40°C for 60 min (Sileks, Russia).

Real-Time Polymerase Chain Reaction. Real-time polymerase chain reaction (PCR) was carried out using the Rotor-Gene Q cycler (Qiagen, Germany). 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 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 45 s. Amplified genes were quantified by fluorescence using the SYBR-Green master mix (Syntol, Russia). After amplification, the specificity of the amplification was monitored using melting curves and agarose gel (1%) electrophoresis. Each sample was run in triplicate, and a non-template control was included in each run. The target gene mRNA expression level was calculated by the efficiency-corrected ΔΔCt method. PCR efficiency was calculated using standard curves corresponding to reference and target genes. The primer sequences are listed in Table 1.

Statistics. Sample volumes were small with non-normal data distributions, and thus the data are expressed as median and interquartile range. The Wilcoxon signed rank test was used to compare the fold change of protein and gene expression with the initial level, and the remaining matched samples were compared using the Wilcoxon matched-pairs test. The relation between samples was evaluated by the Spearman rank correlation test. Level of significance was set at 0.05.

RESULTS

The 1RM was 235 (214−253) N•m. The average exercise intensities for HI, MI, and MIR were 74% (72−75%), 54% (52−55%), and 54% (52−55%), respectively. The average tension time did not differ among groups and was 253 (225−268) s, 262 (242−278) s, and 252 (243−259) s for HI, MI, and MIR, respectively (Fig. 1).

After the third set, blood glucose decreased, but after the sixth and eighth sets, it did not differ from the initial level in all groups. Blood lactate rose ($P < 0.05$) during all exercise sessions, but the increments by which the levels increased were different. Lactate concentration during HI was 2-fold higher ($P < 0.05$) than during MI, but it was greater during MIR than during HI and MI ($P < 0.05$; Fig. 2). Blood insulin, IGF-1, and testosterone levels did not change after any of the exercise sessions (Table 2).

The phosphorylation level of protein kinase B (Akt^{Thr308}) did not change after any of the exercise sessions (Fig. 3). The phosphorylation level of ribosomal protein S6 kinase ($p70S6KThr^{389}$) increased 1.3-fold ($P = 0.048$) at 22 h after termination of MI and did not increase after MIR or HI. HI induced a 1.4-fold increase of 5′-AMP-activated protein kinase $(AMPK)^{Thr172}$ phosphorylation levels 45 min and 22 h after termination of exercise ($P < 0.05$), whereas MIR led to a 1.6-fold increase of $ERK1/2^{Thr202/Tyr204}$ phosphorylation levels 45 min and 22 h after termination of exercise ($P < 0.01$ and $P < 0.05$, respectively). The phosphorylation levels of $AMPK^{Thr172}$ 45 min after HI and of $ERK1/2^{Thr202/Tyr204}$ 45 min and 22 h after MIR were higher ($P < 0.05$) than at those times after MI. A weak but significant correlation was found between peak blood lactate content

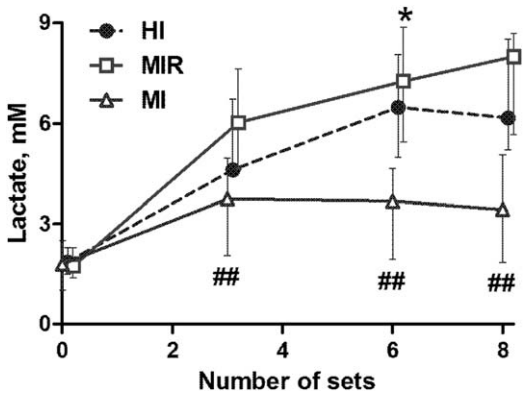

FIGURE 2. Blood lactate levels during high-intensity resistance exercise (HI), middle-intensity exercise (MI), and middle-intensity no-relaxation exercise (MIR). Number of sets is shown on the abscissa. Values expressed as median and interquartile range. $^{*}P < 0.05$ difference vs. HI and $^{\S\S}P < 0.01$ difference vs. MIR and HI.

during exercise and phosphorylation levels of $ERK1/2^{Thr202/Tyr204}$ 45 min after termination of exercise sessions ($r = 0.38$, $P < 0.05$). The data from all exercise sessions were included in the correlation analysis.

The mRNA expression levels of cyclin-dependent kinase inhibitor 1A (p21) and myogenic differentiation 1 (MyoD1), which are markers of satellite cell activation and differentiation, did not change, neither did expression of IGF-1Ea mRNA (Fig. 4). HI induced a 2-fold increase ($P < 0.05$) in MGF (IGF-1Ec) mRNA expression 22 h after termination of the exercise session. Myostatin mRNA expression markedly decreased 22 h after both HI and MIR, by 20-fold ($P < 0.01$) and 6-fold ($P < 0.05$), respectively. After HI and MIR, myostatin mRNA levels were lower ($P < 0.05$) than levels measured after MI.

DISCUSSION

In this study, blood lactate was increased in all exercise sessions, but the increments by which the levels increased were different. The lactate concentration in MI was approximately 2-fold lower than in the 2 other sessions, and lactate concentration was highest in MIR. The latter finding is in agreement with a previous study that compared intramuscular metabolic stress (pH, phosphocreatine, and inorganic phosphate) during MIR (40% of 1RM) and HI (65% of 1RM).[19] The total tension time, total number of contractions, and range of motion were the same in all sessions. Therefore, during MI, the mechanical load and metabolic stress were low. During MIR, the mechanical load was low, but the metabolic stress was the highest and, during HI, the mechanical load was high under pronounced metabolic stress. Thus, the experimental design allowed us to evaluate the effects of resistance exercise intensity and metabolic stress on the activation of intramuscular anabolic signaling and on the expression of myogenic regulatory genes.

Exercise-induced secretion of anabolic hormones depends on the muscle mass involved in exercise. In this study, the testosterone and IGF-1 levels did not change after the exercise sessions, because the working muscle mass was not large. In addition, no changes in insulin level were recorded after the sessions. This finding allowed us to exclude blood hormones as a potential regulator of anabolic signaling and gene expression after the exercise sessions.

Akt is one of the upstream proteins of the Akt-mTORC1 pathway. Previous studies have shown that the Akt^{Thr308} phosphorylation level transiently increased,[24–26] did not change,[27,28] or decreased[29] after resistance exercise. Phosphorylation of Akt is regulated by insulin and IGF-1. In our study, the absence of changes in the phosphorylation of Akt^{Thr308} after exercise may have been associated with the lack of changes in insulin and IGF-1 levels.

One of the mTORC1 downstream targets, phosphorylated $p70S6K^{Thr389}$, was shown to correlate directly with an increase in skeletal muscle mass after resistance exercise.[30,31] In our study, phosphorylation of p70S6K increased 1.3-fold ($P = 0.048$) 22 h after termination of the MI. Increases in exercise intensity and metabolic stress during HI and MIR did not induce an increase of

Table 2. Blood hormones before and 10 min after high-intensity resistance exercise (HI), middle-intensity exercise (MI), and middle-intensity no-relaxation exercise (MIR).

	HI		MIR		MI	
	Before	After	Before	After	Before	After
Insulin (mkU/ml)	20.7 (10.9–39.2)	24.0 (16.3–32.0)	18.3 (11.4–47.3)	19.3 (16.8–28.7)	21.6 (9.6–27.9)	20.3 (12.3–29.6)
IGF-1 (ng/ml)	205 (161–293)	234 (146–270)	227 (175–286)	216 (165–280)	219 (152–278)	212 (143–291)
Testosterone (nmol/L)	12.9 (10.6–15.9)	12.1 (10.0–15.1)	12.5 (10.8–14.5)	10.0 (9.4–14.0)	11.8 (10.6–15.6)	11.8 (10.1–14.9)

Values expressed as the median (interquartile range). IGF-1, insulin-like growth factor-1.

p70S6K^{Thr389} phosphorylation compared with MI. This finding may be related to the training status of our subjects,[27,32] because an increase of phosphorylated p70S6K^{Thr389} content 3 h after HI was found in endurance-trained athletes but not in strength-trained athletes.[33] Chronic strength training in rats also confirmed the assumption that a higher training status leads to a lower increase of the p70S6K^{Thr389} level in response to an acute training session.[32] However, the absence of an increase in p70S6K^{Thr389} phosphorylation level after HI may be connected with activation of AMPK. Indeed, pharmacological activation of AMPK after maximal electrically evoked contractions suppresses mTORC1 signaling in rat skeletal muscle.[34] In our study, the increase in phosphorylated AMPK^{Thr172} level was found only after HI. This finding coincides with the increased activity of AMPKα2[25] and the phosphorylation level of AMPK^{Thr172} during the first hour after HI.[35,36] Adenosine triphosphate (ATP) and phosphocreatine levels are diminished immediately after heavy multiple-set HI.[37] According to the Henneman size principle, the first movements of MI and MIR bouts presumably involve mainly slow-twitch muscle fibers (MFs), because the relative load is substantially lower than 1RM. On the contrary, an HI bout leads to recruitment of both slow- and fast-twitch MFs even during the first movements. Therefore, during the HI bout, fast-twitch MFs recruit earlier than during MI and MIR. The increase in phosphorylation of AMPK 45 min after HI may be connected with fatigue of fast-twitch MFs and decreased ATP content in the muscle immediately after termination of the exercise. Notably, in our study the increase in phosphorylation of AMPK during late recovery may have been connected with more pronounced muscle glycogen depletion after HI when compared with MIR and MI, because total work in HI was approximately 25% higher than in MIR and MI. The decreased muscle glycogen content in the resting state leads to an increase in AMPK activity.[38,39] It is possible that presumed lower muscle glycogen content after HI stimulated AMPK phosphorylation for the recovery period, which lasts up to 22 h.

The ERK1/2 pathway can activate several substrates, such as p90 ribosomal S6 kinase (p90S6K) and MAPK-interacting kinase 1, which leads to activation of the ribosomal subunit S6 and transcription factors.[40] Most studies have reported an

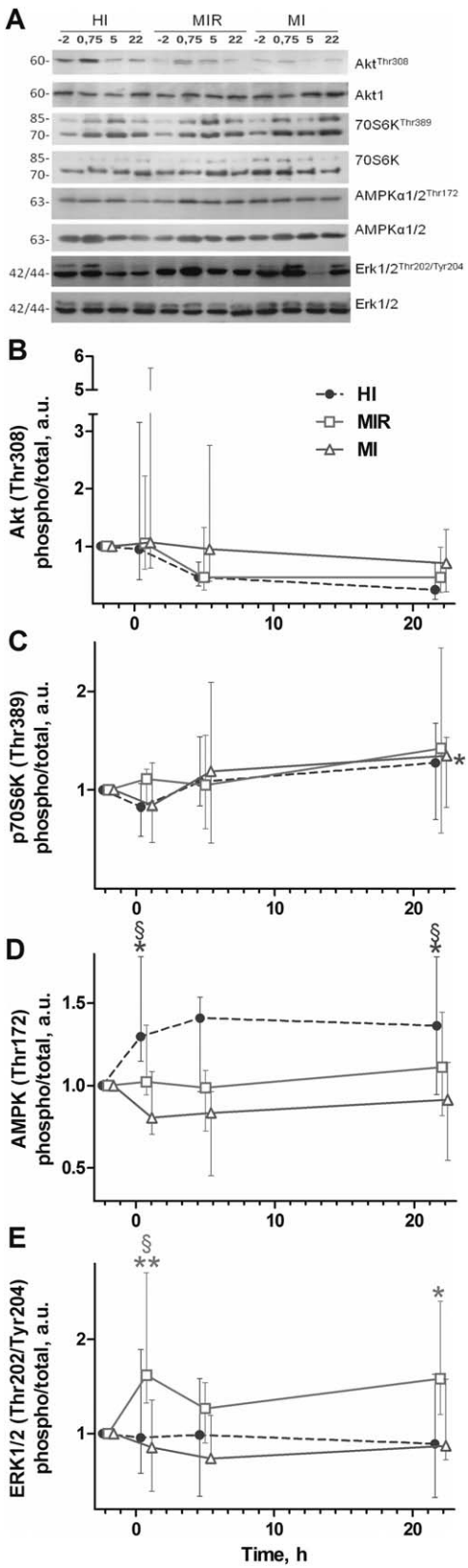

FIGURE 3.

FIGURE 3. (A) Representative immunoblot. The fold change of phosphorylation levels (phosphorylated to total protein) of **(B)** Akt, **(C)** p70S6K, **(D)** AMPK, and **(E)** ERK1/2 before and after high-intensity resistance exercise (HI), middle-intensity exercise (MI), and middle-intensity no-relaxation exercise (MIR) normalized to the initial level. The time of exercise session termination is 0 h. Values are expressed as median and interquartile range. $^{*}P < 0.05$ difference vs. the initial level; $^{**}P < 0.01$ difference vs. initial level; and $^{\S}P < 0.05$ difference vs. MI.

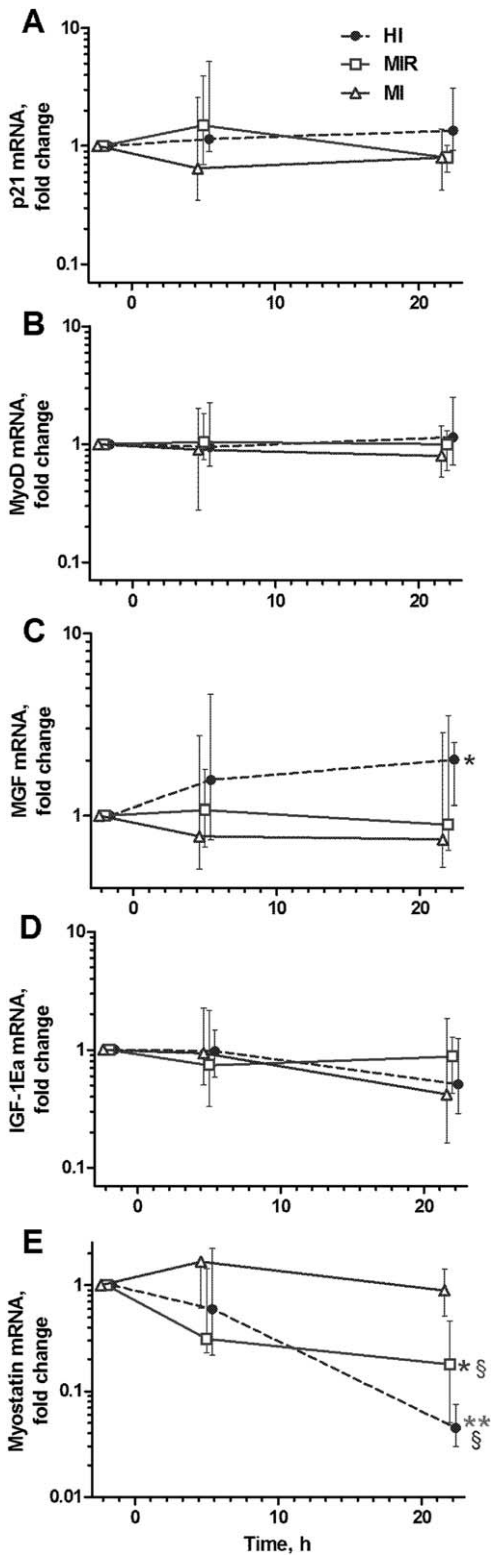

FIGURE 4. The fold change of the mRNA level of **(A)** p21, **(B)** MyoD, **(C)** MGF (IGF-1Ec), **(D)** IGF-1Ea, and **(E)** myostatin, before and after high-intensity resistance exercise (HI), middle-intensity exercise (MI), and middle-intensity no-relaxation exercise (MIR). The time of exercise session termination is 0 h. Values expressed as median and interquartile range. $^{*}P < 0.05$ difference vs. initial level; $^{**}P < 0.01$ difference vs. initial level; and $^{\S}P < 0.05$ difference vs. MI.

increase of ERK1/2$^{Thr202/Tyr204}$ phosphorylation immediately after or during the first hour after HI.[41,42] However, 1 study indicated an increase at 6 h and 24 h after exercise.[26] The increase of ERK1/2$^{Thr202/Tyr204}$ and p90S6K^{Thr573} phosphorylation was shown to not depend on the degree of muscle glycogen depletion[24] or on the adaptation of muscle to strength training.[27,32] We showed that the increase of exercise intensity from 54% to 74% 1RM did not induce change in the phosphorylation level of ERK1/2$^{Thr202/Tyr204}$. This finding may be connected with the AMPK activation, because the pharmacological increase of AMPKα1/α2 activity in the myotube blocked the increase of the ERK1/2$^{Thr202/Tyr204}$ phosphorylation level, presumably through Raf1.[43] However, the substantial metabolic stress increased the ERK1/2 phosphorylation level 45 min and 22 h after MIR. This finding coincides with the increase found in ERK1/2$^{Thr202/Tyr204}$ and p90S6K^{Thr573} phosphorylation levels at 4 h and 24 h, respectively, after a low-intensity (30% 1RM) exercise that was performed without pause until volitional failure.[6,28] Interestingly, this type of exercise was similar to the MIR exercise regime in our study. The phosphorylation of ERK1/2 is expected to be sensitive to the number of contractions performed during an exercise bout.[42] In our study, the number of contractions was the same in all sessions. Therefore, activation of ERK1/2$^{Thr202/Tyr204}$ depended on metabolic stress (comparison of the MIR and MI). This finding is consistent with the result of a previous study in isolated rat skeletal muscle.[4] The MIR-induced increase of the ERK1/2 phosphorylation level may be connected to increased reactive oxygen species (ROS) production, because MIR induced repeated episodes of ischemia–reperfusion. ROS may then have interacted with acidosis and mechanical tension to cause a greater response to the ERK1/2 phosphorylation level than in the other protocols.[4] In the MIR, the working muscle oxygenation index is substantially decreased in comparison to HI and MI.[13] A myoblast study showed that hypoxia enhances and prolongs ERK1/2$^{Thr202/Tyr204}$ activation in a hypoxia-inducible factor-1 (HIF-1)-dependent fashion.[44] Based on our study findings, we suggest that the increase in ERK1/2 phosphorylation level at the later stage of recovery after MIR was related to activation of HIF-1. To our knowledge, there are no existing studies on HIF-1 protein expression after resistance exercise. However, Larkin *et al.*[45] showed that MIR (40% of 1RM) induced expression of HIF-1 mRNA, whereas traditional resistance exercise with the same intensity did not induce changes in expression of this gene.

Satellite cells are important for resistance exercise–induced muscle hypertrophy.[46] We did not

observe changes in the mRNA expression of p21 and MyoD1, which are the markers of satellite cell activation and differentiation. It has been suggested that, in mature skeletal muscle, MGF (IGF-1Ec) is responsible for satellite cell activation and proliferation, whereas IGF-1Ea is responsible for differentiation.[47] We found that only HI induced MGF (IGF-1Ec) mRNA expression 22 h after termination of the exercise. This finding allows us to conclude that mRNA expression of MGF (IGF-1Ec) in trained muscle depends on the exercise intensity (comparison of MI and HI) and does not depend on the metabolic stress (comparison of MIR and MI). We have shown previously that myofibrillar proteins (such as myomesin 1, myosin-binding protein C, and titin) released from damaged cells stimulate MGF expression at both the mRNA and protein levels in primary murine myoblasts or differentiated *in vitro* myotubes.[48] It is possible to speculate that, in our study, HI induced degradation of myofibrillar proteins, which may stimulate *MGF* gene expression. This suggestion is supported by our previous data. HI leg-press exercise sessions increased blood creatine kinase activity (marker of muscle cell damage) at 20 h of recovery more than MIR.[11] In skeletal muscle, resistance exercise−induced expression of MGF (IGF-1Ec) mRNA occurs earlier (at 24 h) compared with IGF-1Ea mRNA (at 72 h).[49] This finding may explain partially the lack of changes in IGF-1Ea mRNA expression in our study.

Myostatin inhibits muscle stem cell proliferation[50] and differentiation by down-regulating MyoD expression,[51] and it activates the forkhead box protein (FOXO)-E3 ligase−proteasome system through down-regulation of Akt.[52] We found that high-intensity exercise under metabolic stress (HI) decreased (∼20-fold) the myostatin mRNA level. Comparison of MI and MIR allowed us to conclude that substantial metabolic stress is a sufficient stimulus to decrease (∼6-fold) myostatin mRNA expression.

Limitations. There are some notable limitations to this study. In the MIR, each repetition was followed immediately by another, so an eccentric phase preceded a concentric phase for each new repetition as in a stretch-shortening cycle (SSC). Potentially it may increase skeletal muscle mechanical efficiency during the concentric phase. Also, eccentric knee angle velocity was relatively low (30°/s) in all protocols, and therefore potentiation effects of the slow SSC movements may be minimal due to the prolonged eccentric phase.[53]

The mTORC1, MAPK, and FAK signaling pathways and strength training−induced muscle hypertrophy have been shown to depend on different contraction variables such as peak torque, the time−torque integral, and rate of strain.[54,55] Our study design did not allow us to evaluate the influence of these variables on muscle anabolic signaling.

The expression of myogenic factors was investigated at the mRNA level only. Despite the lack of change in *p21* gene and *MyoD1* gene expression, it is possible that satellite cell activation and proliferation occurred (but this was not detected) along with possible differences between the protocols.

CONCLUSION

Our study and other recent works[6,7,28] have demonstrated that contractile variables, such as intensity, duration, work, and exercise-induced metabolic stress, can be manipulated to affect the responses of muscle anabolic signaling. We showed that, in trained skeletal muscle, the phosphorylation level of ERK1/2$^{Thr202/Tyr204}$ after resistance exercise was related to metabolic stress and did not depend on exercise intensity. Metabolic stress itself induced a decrease in myostatin mRNA expression, whereas MGF (IGF-1Ec) mRNA level depended on resistance exercise intensity and not on metabolic perturbations.

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