Resistance Exercise Increases Postprandial Muscle Protein Synthesis in Humans

OLIVER C. WITARD[1], MICHAEL TIELAND[2], MILOU BEELEN[2], KEVIN D. TIPTON[1], LUC J. C. VAN LOON[2], and RENÉ KOOPMAN[2]

[1]*School of Sport and Exercise Sciences, University of Birmingham, Birmingham, UNITED KINGDOM; and* [2]*Department of Human Movement Sciences, NUTRIM, Maastricht University, Maastricht, THE NETHERLANDS*

ABSTRACT

WITARD, O. C., M. TIELAND, M. BEELEN, K. D. TIPTON, L. J. VAN LOON, and R. KOOPMAN. Resistance Exercise Increases Postprandial Muscle Protein Synthesis in Humans. *Med. Sci. Sports Exerc.*, Vol. 41, No. 1, pp. 144–154, 2009. **Purpose**: We examined the impact of an acute bout of resistance-type exercise on mixed muscle protein synthesis in the fed state. **Methods**: After a standardized breakfast, 10 untrained males completed a single, unilateral lower-limb resistance-type exercise session. A primed, continuous infusion of L-[ring-$^{13}C_6$]phenylalanine was combined with muscle biopsy collection from both the exercised (Ex) and the nonexercised (NEx) leg to assess the impact of local muscle contractions on muscle protein synthesis rates after food intake. Western blotting with phosphospecific and pan antibodies was used to determine the phosphorylation status of AMP-activated kinase (AMPK), 4E-binding protein (4E-BP1), mammalian target of rapamycin (mTOR), and p70 ribosomal protein S6 kinase (S6K1). **Results**: Muscle protein synthesis rates were approximately 20% higher in Ex compared with NEx (0.098% ± 0.005% vs 0.083% ± 0.002%·h^{-1}, respectively, $P < 0.01$). In the fed state, resistance-type exercise did not elevate AMPK phosphorylation. However, the phosphorylation status of 4E-BP1 was approximately 20% lower after cessation of exercise in Ex compared with NEx ($P < 0.05$). Conversely, 4E-BP1 phosphorylation was significantly higher in Ex compared with NEx after 6 h of recovery ($P < 0.05$) with no changes in mTOR phosphorylation. S6 phosphorylation was greater in Ex versus NEx after cessation of exercise ($P < 0.05$), although S6K1 phosphorylation at T^{389} was not up-regulated ($P > 0.05$). **Conclusion**: We conclude that resistance-type exercise performed in a fed state further elevates postprandial muscle protein synthesis rates, which is accompanied by an increase in S6 and 4E-BP1 phosphorylation state. **Key Words:** SKELETAL MUSCLE, FOOD INTAKE, TRANSLATION INITIATION, LOCAL MUSCLE CONTRACTION

BASIC SCIENCES

Skeletal muscle protein synthesis is accelerated after an acute bout of resistance-type exercise (9,28). However, in the absence of nutrient intake, net muscle protein balance (synthesis–breakdown) remains negative (3,28,29). Numerous studies unequivocally demonstrate that the ingestion of essential amino acids (8,35) and/or intact protein (22,34) can further stimulate muscle protein synthesis rates after resistance-type exercise performed in a fasted state, resulting in net muscle protein accretion. However, with the experimental setup of these studies, it is not possible to differentiate between exercise- versus diet-induced protein synthetic response.

The application of a unilateral leg exercise protocol, with the contralateral leg as a control, has been shown to provide an appropriate tool to study the additive effect of exercise on muscle protein synthesis (29,32). Using such an approach, it has been shown that resistance exercise training results in an altered response to an acute bout of resistance exercise followed by food intake (32). However, to date, no study has examined the impact of resistance-type exercise on muscle protein synthesis after meal ingestion using the unilateral exercise model.

As changes in muscle protein synthesis occur before changes in muscle mRNA content (38), it is commonly accepted that muscle protein synthesis is largely controlled on a posttranscriptional level. The initiation of mRNA translation, which includes the binding of the initiator methionyl–tRNA and mRNA to the ribosomal subunits, is generally regarded to be rate limiting (18). Much recent research has been focused on the role played by the mammalian target of rapamycin (mTOR) signal pathway, that is, activation of mTOR and its downstream signaling proteins p70 ribosomal protein S6 kinase (S6K1) and eukaryotic initiation factor (eIF) 4E-binding protein (4E-BP1), in regulating translation initiation (18). Both S6K1 and 4E-BP1 modulate translation initiation and control the binding of

Address for correspondence: René Koopman, M.D., Department of Human Movement Sciences, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands; E-mail: R.Koopman@HB.unimaas.nl.
Submitted for publication May 2008.
Accepted for publication June 2008.

0195-9131/09/4101-0144/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e3181844e79

TABLE 1. Subjects' characteristics.

	Subjects
Age (yr)	22.4 ± 0.7
Weight (kg)	76.8 ± 2.3
Height (m)	1.85 ± 0.02
BMI ($kg \cdot m^{-2}$)	22.4 ± 0.5
% body fat	17.7 ± 1.7
Basal glucose ($mmol \cdot L^{-1}$)	5.1 ± 0.1
Basal insulin ($mU \cdot L^{-1}$)	10.3 ± 0.8
Leg volume (L)	8.8 ± 0.4
Single-leg 1-RM leg press (kg)	127 ± 7
Single-leg 1-RM leg extension (kg)	63 ± 4

Values are expressed as means ± SEM (n = 10). 1-RM, one repetition maximum.

mRNA to the 40S ribosomal subunit. 4E-BP1 can bind to the initiation factor eIF4E, thereby acting as a translational repressor, thus preventing the formation of the eIF4F scaffolding complex that is necessary for efficient binding of the 40S ribosomal subunit to mRNA (18). Via mTOR-mediated phosphorylation of 4E-BP1, 4E-BP1 is released from the initiation factor eIF4E, allowing the formation of the active eIF4F complex that mediates binding of mRNA to the 40S ribosomal unit, consequently allowing the translation–initiation process to occur (18). Another mechanism regulating the binding of mRNA to the 40S ribosomal subunit involves the phosphorylation of ribosomal protein S6 (S6), which is controlled by the activity of S6K1. Activation of S6K1 leads to the phosphorylation of S6 on the 40S subunit, located in near proximity to the eIF. As a consequence, the interaction of the ribosomal subunit protein with the mRNA molecule promotes mRNA translation.

Pioneering studies in rodents demonstrate increased phosphorylation of both 4E-BP1 and S6K1 in response to electrical stimulation of the muscle (1,4,26), resistance-type exercise (24), and protein and/or leucine intake (18). Only a few studies have measured muscle protein synthesis and intracellular signaling simultaneously after exercise and nutrition in humans (10,12,13,16). Therefore, evidence linking accelerated protein synthetic rates after exercise and nutrition in humans with the activation of the mTOR pathway remains limited.

The aim of the present study is to determine the impact of resistance-type exercise on muscle fractional synthetic rate (FSR) and mTOR-associated signaling proteins in a setting in which resistance-type exercise is performed in the fed state. A single-leg exercise protocol was performed to allow differentiation between the muscle protein synthetic response to local muscle contraction and the systemic changes in substrate availability and hormonal response.

METHODS

Subjects. Ten healthy male volunteers with no history of participating in any regular exercise program were recruited to participate in the present study. Subjects' characteristics are provided in Table 1. All subjects were informed on the nature and the possible risks of the experimental procedures before written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht. All volunteers were instructed to refrain from any sort of heavy physical exercise and to consume a normal diet for 3 d before the experiment.

Pretesting. Body composition was assessed using the hydrostatic weighing method in the morning after an overnight fast as described previously (23). Thereafter, maximum single-leg strength was estimated using the multiple repetitions testing procedure for leg press and leg extension (23). In an additional exercise session, at least 1 wk before the first experimental treatment, the subjects' single-leg one repetition maximum (1-RM) was determined (23).

Protocol. The evening before the experiment, subjects consumed a standardized meal (32 ± 2 $kJ \cdot kg^{-1}$ body weight, consisting of 55 energy percent (En%) carbohydrate, 25 En% protein, and 30 En% fat). A schematic overview of the study protocol is provided in Figure 1. At

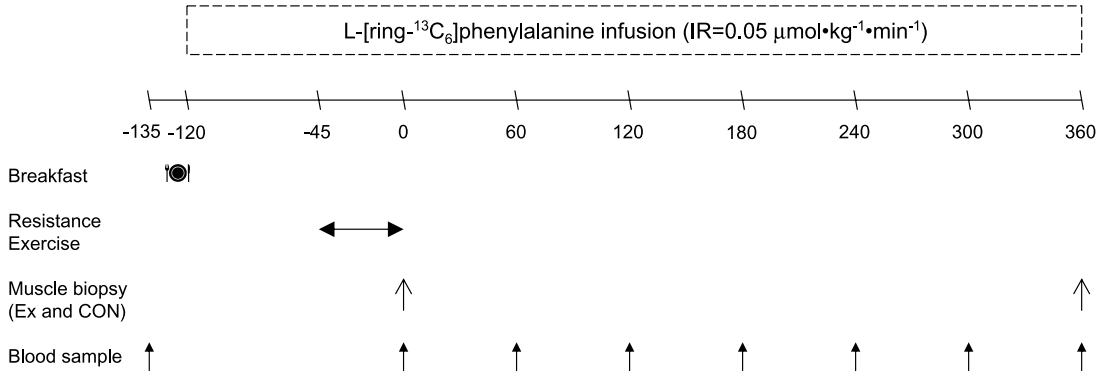


FIGURE 1—Schematic representation of the experimental protocol. Muscle biopsies were collected from the exercised (Ex) and the nonexercised (NEx) leg immediately after cessation of exercise and after 6 h of postexercise recovery. Time is expressed in minutes before or after the exercise bout. IR, infusion rate.

TABLE 2. Plasma and muscle tracer L-[ring-$^{13}C_6$]phenylalanine enrichments during postexercise recovery.

	Plasma (n = 10)	Ex Leg (n = 10)	NEx Leg (n = 10)
Enrichment during recovery (TTR)	0.0687 ± 0.0026	0.0507 ± 0.0020*	0.0438 ± 0.0019
Δ Enrichment muscle protein (TTR)	NA	0.00030 ± 0.00001*	0.00022 ± 0.00001

Values represent means ± SEM. Tracer enrichments are expressed as tracer/tracee ratio (TTR). NA, not applicable.
* Significantly different from NEx leg ($P < 0.05$).

8:00 a.m., after an overnight fast, subjects arrived at the laboratory by car or by public transportation. A Teflon catheter was inserted into an antecubital vein for stable isotope infusion. A second Teflon catheter was inserted into a heated dorsal hand vein of the contralateral arm and placed in a hotbox (60°C) for arterialized blood sampling. After basal blood sample collection, subjects consumed a standardized breakfast (31 ± 1 kJ·kg^{-1} body weight, consisting of 52 energy percent (En%) carbohydrate, 34 En% protein, and 14 En% fat) containing 50 g breakfast cereal (Quaker Cruesli; PepsiCo, Utrecht, The Netherlands), 500 g curd cheese (Zaanse Hoeve; Campina, Veghel, The Netherlands), and 10 mL strawberry flavoring (Karvan Cévitam; Heinz, Utrecht, The Netherlands). Thereafter (t = −120 min), a single intravenous dose of L-[ring-$^{13}C_6$]phenylalanine (2 μmol·kg^{-1}) was administered to prime the phenylalanine pool. Subsequently, continuous tracer infusion was started [infusion rate = 0.049 ± 0.001 μmol·kg^{-1}·min^{-1}] for L-[ring-$^{13}C_6$]phenylalanine. Subjects rested in a supine position for 1 h before engaging in the resistance exercise protocol. After a 5-min warm-up on a cycle ergometer (~75 W), subjects completed a session of unilateral lower-limb exercises consisting of eight sets of

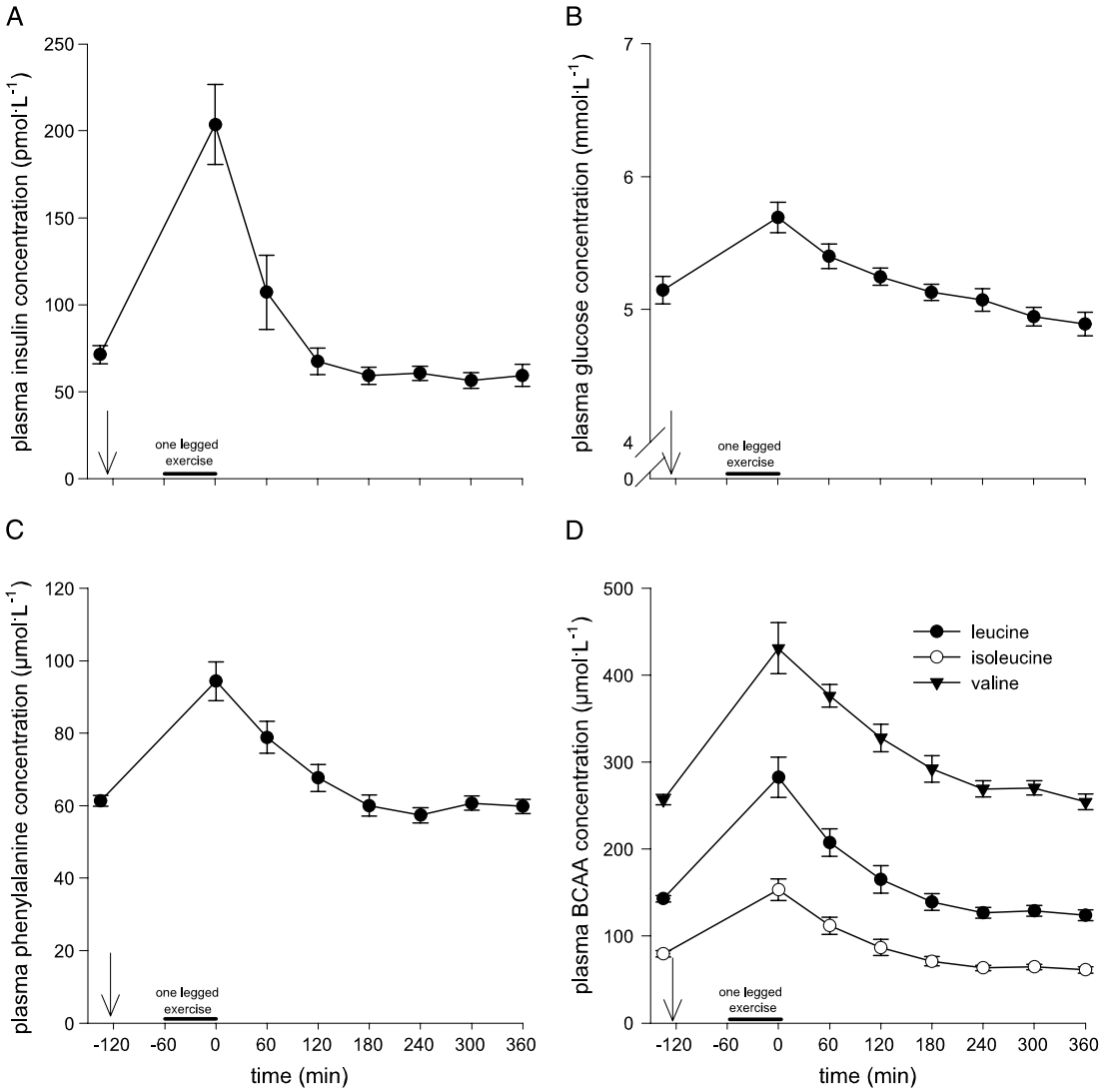


FIGURE 2—Plasma insulin (*A*), glucose (*B*), phenylalanine (*C*), and leucine, isoleucine, and valine concentrations (*D*) during the entire test period. The horizontal lines in the graphs indicate the time period of the single-leg exercise. Values represent means ± SEM (n = 10). Arrows indicate timing of the breakfast.

10 repetitions on the leg press and leg extension machines (Technogym BV, Rotterdam, The Netherlands), both performed at 70% of their individual 1-RM with 2-min rest intervals between sets. All subjects were verbally encouraged during exercise, and the entire exercise protocol required approximately 45 min to complete. At the end of the exercise protocol (t = 0 min), subjects rested supine and an arterialized blood sample and muscle biopsies from the vastus lateralis muscle of the exercised (Ex) and the nonexercised (NEx) leg were collected. Arterialized blood samples were collected at t = 60, 120, 180, 240, 300, and 360 min with additional muscle biopsies taken at t = 360 min from both the Ex and the NEx limb, each from a new incision.

Biopsies were obtained from the middle region of the vastus lateralis muscle (~15 cm above the patella) and approximately 3 cm below entry through the fascia using the percutaneous needle biopsy technique (2). Muscle samples were dissected carefully and freed from any visible nonmuscle material. The muscle sample was immediately frozen in liquid nitrogen and stored at −80°C until further analysis. Blood samples were collected in EDTA containing tubes and centrifuged at 1000*g* and 4°C for 5 min. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C until further analyses.

Plasma sample analyses. Plasma glucose (Uni KitIII, 07367204; Roche, Basel, Switzerland) concentrations were analyzed with the COBAS-FARA semiautomatic analyzer (Roche). Insulin was analyzed by radio immunoassay (Insulin RIA kit; LINCO Research Inc., St. Charles, MO). Plasma (100 μL) was deproteinized using 5-sulphosalicylic acid, and free amino acid concentrations were measured using a high-performance liquid chromatography technique after precolumn derivatization with *o*-phthalaldehyde (36). For measurement of plasma phenylalanine enrichment, plasma phenylalanine was derivatized

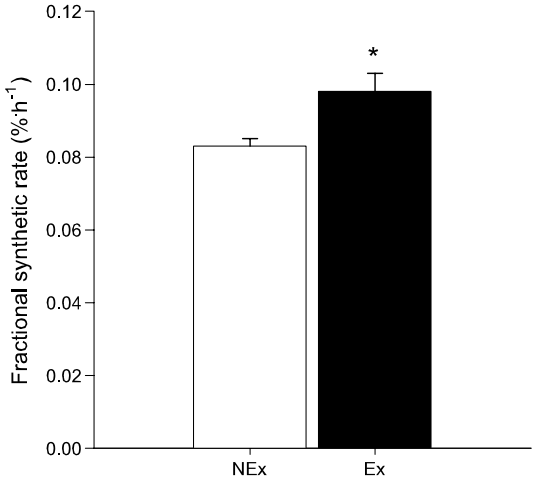


FIGURE 3—Mean (±SEM) fractional synthetic rate (FSR) of mixed muscle protein during postexercise recovery in the nonexercised (NEx) and the exercised (Ex) leg in lean, young males (n = 10) with the use of plasma phenylalanine enrichment as a precursor. * Significantly different from the NEx leg, $P < 0.05$.

to its *t*-butyldimethylsilyl derivative, and ^{13}C enrichment was determined by electron ionization gas chromatography–mass spectrometry (Agilent 6890N GC/5973N MSD, Little Falls, DE) using selected ion monitoring of masses 336 and 342 for unlabeled and labeled phenylalanine, respectively (22). We applied standard regression curves in all isotopic enrichment analysis to assess linearity of the mass spectrometer and to control for loss of tracer.

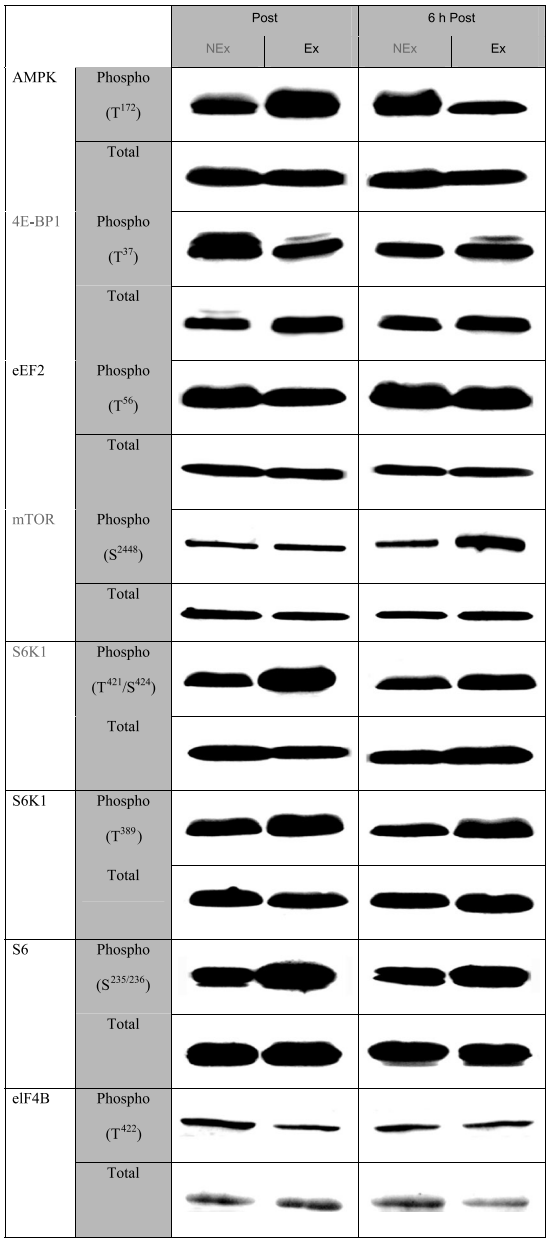
Muscle sample analyses. For measurement of L-[ring-$^{13}C_6$]phenylalanine enrichment in the free amino acid pool and mixed muscle protein, 55 mg of wet muscle was freeze dried. Collagen, blood, and other nonmuscle fiber material were removed from the muscle fibers under a light microscope. The isolated muscle fiber mass (10 mg) was weighed, and eight volumes (8× dry weight of isolated

TABLE 3. Muscle free amino acid concentrations during postexercise recovery.

	NEx Leg (n = 10)			Ex Leg (n = 10)		
	t = 0	t = 360	(0–360)	t = 0	t = 360	(0–360)
GLU	2536 ± 327	2465 ± 319	−71 ± 470	1939 ± 298	3449 ± 407	1510 ± 267*
ASN	208 ± 14	152 ± 11	−55 ± 14	221 ± 15	201 ± 18	−20 ± 17
SER	542 ± 22	440 ± 13	−102 ± 424	541 ± 33	540 ± 31	−1 ± 30*
GLN	9468 ± 830	7362 ± 410	−2106 ± 939	8746 ± 720	10,403 ± 1094	1658 ± 705*
HIS	393 ± 34	316 ± 15	−77 ± 31	356 ± 27	428 ± 407	72 ± 35*
GLY	836 ± 65	784 ± 135	−52 ± 153	831 ± 74	887 ± 87	56 ± 46
THR	518 ± 38	398 ± 19	−120 ± 34	557 ± 43	536 ± 58	−21 ± 41
CIT	84 ± 9	70 ± 6	−14 ± 7	84 ± 8	103 ± 13	19 ± 7*
ARG	311 ± 28	220 ± 190	−91 ± 32	278 ± 26	254 ± 44	−24 ± 31
ALA	1824 ± 178	971 ± 109	−853 ± 245	1589 ± 142	1116 ± 115	−473 ± 175*
TYR	143 ± 11	87 ± 13	−56 ± 12	139 ± 14	78 ± 7	−61 ± 15
VAL	279 ± 17	236 ± 18	−43 ± 15	305 ± 23	245 ± 21	−59 ± 22
MET	30 ± 9	14 ± 3	−17 ± 6	26 ± 6	16 ± 4	−13 ± 5
ILE	100 ± 9	70 ± 11	−30 ± 21	111 ± 12	67 ± 10	−39 ± 21
PHE	68 ± 7	61 ± 8	−7 ± 3	72 ± 7	50 ± 5	−22 ± 8
TRP	16 ± 2	11 ± 3	8 ± 2	15 ± 2	17 ± 7	3 ± 6
LEU	184 ± 12	139 ± 15	−45 ± 12	199 ± 18	132 ± 12	−68 ± 19
ORN	171 ± 13	146 ± 10	−24 ± 15	142 ± 11	164 ± 15	21 ± 11*
LYS	184 ± 12	139 ± 15	−45 ± 12	199 ± 18	132 ± 12	−168 ± 19

Values represent means ± SEM. Data are expressed in nmol·g^{-1} wet muscle weight.
* Significantly different from NEx leg ($P < 0.05$).

TABLE 4. Representative blots of phosphorylated and total protein content.



NEx, nonexercised leg; Ex, exercised leg.

muscle fibers × wet/dry ratio) of ice-cold 2% perchloric acid were added. The tissue was then homogenized and centrifuged. The supernatant was collected and processed in the same manner as the plasma samples, such that intracellular free L-[ring-$^{13}C_6$]phenylalanine enrichments could be measured using their *t*-butyldimethylsilyl derivatives on a gas chromatography–mass spectrometry (22) and free amino acid concentration could be measured using a high-performance liquid chromatography technique (36).

The protein pellet was washed with three additional 1.5-mL washes of 2% perchloric acid, dried, and the proteins were hydrolyzed in 6 M HCl at 120°C for 15 to 18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while heated to 120°C then dissolved in a 50% acetic acid solution and passed over a Dowex exchange resin (AG 50W-X8, 100–200 mesh hydrogen form; Bio-Rad, Hercules, CA) using 2 M NH_4OH. Thereafter, the eluate was dried, and the purified amino acids were derivatized to their *N*(*O*,*S*)-ethoxycarbonyl ethyl esters for the determination of $^{13}C/^{12}C$ ratios of muscle protein-bound phenylalanine (19). Thereafter, the derivative was measured by GC-IRMS (Finnigan MAT 252, Bremen, Germany) using the HP Ultra I GC-column (#19091A-112; Hewlett-Packard, Palo Alto, CA) combustion interface II and monitoring of ion masses 44, 45, and 46. By establishing the relationship between the enrichment of a series of [ring-$^{13}C_6$]phenylalanine standards of variable enrichment and the enrichment of the *N*(*O*,*S*)-ethoxycarbonyl ethyl esters of these standards, the muscle protein-bound enrichment of phenylalanine was determined (Table 2). We applied standard regression curves to assess linearity of the mass spectrometer and to control for loss of tracer. The coefficient of variance (CV) for the measurement of L-[ring-$^{13}C_6$] phenylalanine enrichment in mixed muscle protein averaged 4.3% ± 0.6%.

Another portion of the muscle samples was treated and homogenized using a previously described buffer containing several protease inhibitors (7,20,23). Primary phospho-specific antibodies [antiphospho–AMP-activated kinase (AMPK) (T^{172}), antiphospho–4E-BP1 (T^{37}) antiphospho-eEF2 (T^{56}), antiphospho-mTOR (S^{2448}), antiphospho-S6K1 (T^{421}/S^{424}), antiphospho-S6K1 (T^{389}), antiphospho-S6 ($S^{235/236}$), and antiphospho-eIF4B (T^{422})], and anti-AMPK, anti–4E-BP1, anti-eEF2, anti-mTOR, anti-S6K1, anti-S6, and anti-eIF4B were purchased from Cell Signaling Technologies (Beverly, MA). Quantification of phosphorylation status of AMPK, 4E-BP1, eEF2, mTOR, S6K1, S6, and eIF4B was performed using Western blotting with phosphospecific and aspecific antibodies as previously described using α-actin as a loading control (20,23). Phosphorylation was expressed relative to the total amount of each protein.

Calculations. Fractional synthetic rate (FSR) of mixed muscle protein was calculated by dividing the increment in enrichment in the product, that is, protein-bound L-[ring-$^{13}C_6$]phenylalanine, by the enrichment of the precursor. Free muscle L-[ring-$^{13}C_6$]phenylalanine enrichment was used as precursor pool to calculate fractional synthesis rate of mixed muscle protein (22).

Statistics. The present study was designed to assess the impact of single-leg exercise on muscle protein synthesis during recovery. All data are expressed as means ± SEM. Two-way repeated-measures ANOVA were applied to determine differences in phosphorylation status in the proteins of interest over time between treatments. In case of significant interaction between time and treatment effects, Student paired *t*-tests were applied to locate differences between treatments and biopsies taken immediately postexercise and after 6 h of recovery. Statistical significance was set at $P < 0.05$. All calculations

were performed using StatView 5.0 (SAS Institute Inc., Cary, NC).

RESULTS

Plasma insulin, glucose, and amino acid concentrations. Plasma insulin, glucose, phenylalanine, and BCAA (leucine, isoleucine, and valine) concentrations reached maximal values after cessation of exercise (~2 h after breakfast) and returned to baseline values within the next 2 to 4 h (Fig. 2).

Muscle phenylalanine enrichment and amino acid concentrations. Free muscle L-[ring-$^{13}C_6$] phenylalanine enrichment and L-[ring-$^{13}C_6$]phenylalanine incorporation were significantly higher in the exercised (Ex) leg compared with the nonexercised (NEx) leg (Table 2). Free muscle phenylalanine, leucine, isoleucine, and valine concentrations as determined in the 0- and the 6-h post-exercise muscle biopsies did not differ between the Ex and the NEx leg (Table 3).

Muscle protein synthesis. Mixed muscle protein fractional synthetic rate (FSR), with the average free L-[ring-$^{13}C_6$]phenylalanine enrichment in muscle tissue collected after exercise (t = 0 min) and 6 h into recovery (t = 360 min) as the precursor pool, averaged 0.083% ± 0.002% and 0.098% ± 0.005%·h^{-1} in the NEx and the Ex leg, respectively (Fig. 3; $P < 0.05$).

Western blotting results. Representative blots of phosphorylated and total protein content of the signaling proteins are presented in Table 4. The changes in the phosphorylation status of AMPK (T^{172}), 4E-BP1 (T^{37}), eEF2 (T^{56}), and mTOR (S^{2448}) during recovery from single-legged

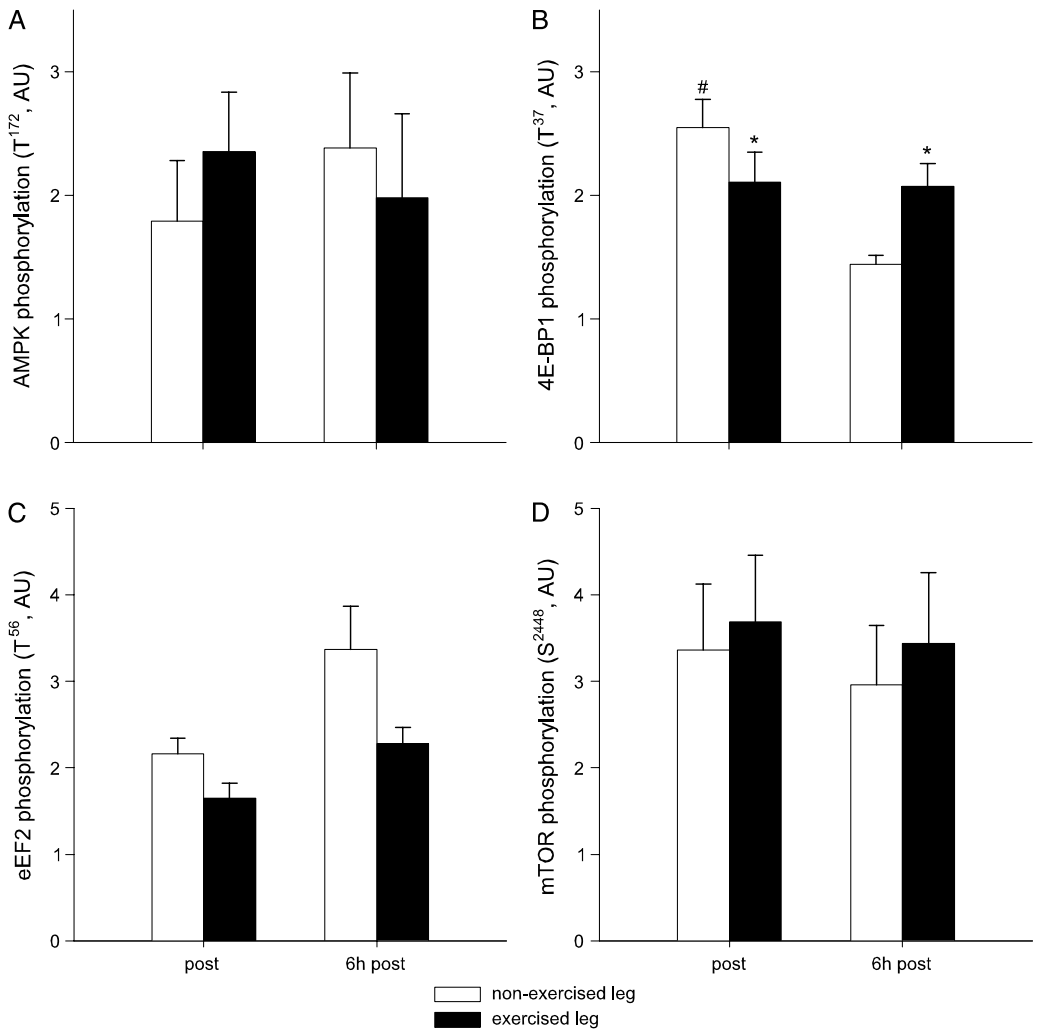


FIGURE 4—AMPK phosphorylation at T^{172} (*A*), 4E-BP1 phosphorylation at T^{37} (*B*), eEF2 phosphorylation at T^{56} (*C*), and mTOR phosphorylation at S^{2448} (*D*) immediately after cessation of exercise and after 6 h of postexercise recovery in the Ex and the NEx leg. Representative immunoblots are shown (*top*). Values represent means ± SEM (n = 10). AMPK (T^{172}): treatment effect, P = 0.75; time effect, P = 0.22; interaction of treatment and time, P = 0.41. 4E-BP1 (T^{37}): treatment effect, P = 0.68; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. eEF2 (T^{56}): treatment effect, P = 0.87; time effect, P = 0.25; interaction of treatment and time, P = 0.10. mTOR (S^{2448}): treatment effect, P = 0.79; time effect, P = 0.22; interaction of treatment and time, P = 0.41. * Significantly different from the NEx leg, $P < 0.05$. # Significantly different from values at 6 h post, $P < 0.05$.

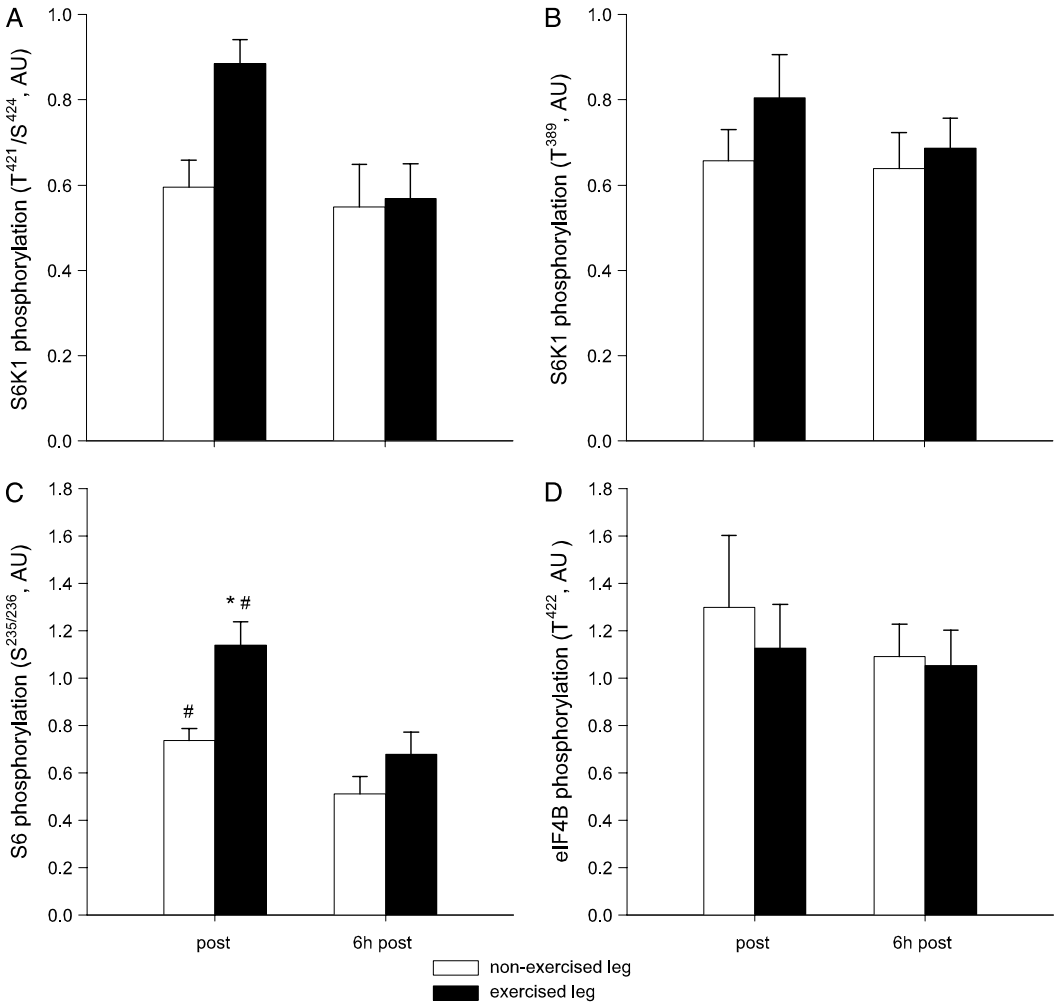


FIGURE 5—S6K1 phosphorylation at T^{421}/S^{424} (*A*), S6K1 phosphorylation at T^{389} (*B*), S6 phosphorylation at $S^{235/236}$ (*C*), and eIF4B phosphorylation at S^{422} (*D*) immediately after cessation of exercise and after 6 h of postexercise recovery in the Ex and the NEx leg. Representative immunoblots are shown (*top*). Values represent means ± SEM (n = 10). S6K1 (T^{421}/S^{424}): treatment effect, $P = 0.06$; time effect, $P < 0.05$; interaction of treatment and time, $P = 0.12$. S6K1 (T^{389}): treatment effect, $P = 0.23$; time effect, $P = 0.40$; interaction of treatment and time, $P = 0.63$. S6 ($S^{235/236}$): treatment effect, $P < 0.05$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.05$. eIF4B (S^{422}): treatment effect, $P = 0.87$; time effect, $P = 0.25$; interaction of treatment and time, $P = 0.59$. * Significantly different from NEx leg, $P < 0.05$. # Significantly different from values at 6 h post, $P < 0.05$.

resistance-type exercise in the Ex and the NEx leg are presented in Figure 4. No significant differences in phosphorylation status of AMPK, eEF2, and mTOR were observed between the NEx and the Ex leg after cessation of exercise or after 6 h of postexercise recovery. 4E-BP1 phosphorylation was significantly lower in the Ex leg compared with the NEx leg immediately after exercise ($P < 0.05$). Over time, a reduction in 4E-BP1 phosphorylation was observed in the NEx leg, whereas no changes over time were observed in the Ex leg (Fig. 4B). As a result, 4E-BP1 phosphorylation was significantly higher in muscle taken from the Ex leg at 6 h postexercise when compared with the NEx leg ($P < 0.05$).

The changes in the phosphorylation status of S6K1 (T^{421}/S^{424} and T^{389}), S6 ($S^{235/236}$), and eIF4B (S^{422}) during recovery from single-legged resistance exercise in the Ex and the NEx leg are presented in Figure 5. Two-way repeated-measures ANOVA revealed a significant time effect ($P < 0.05$) and a trend for a treatment effect ($P = 0.06$) without significant interaction ($P = 0.12$) for S6K1 phosphorylation status (T^{421}/S^{424}). No significant changes in phosphorylation status of S6K1 (T^{389}) and eIF4B (S^{422}) were observed between the NEx and the Ex leg. S6 phosphorylation status was significantly higher immediately after cessation of exercise in the Ex leg compared with the NEx leg ($P < 0.05$). During recovery, the S6 phosphorylation status declined in both the NEx and the Ex leg (−42% ± 7% vs −26% ± 10%, respectively, P = NS). No significant differences in S6 phosphorylation were observed after 6 h of recovery.

DISCUSSION

In the present study, we assessed the impact of resistance exercise on skeletal muscle protein synthesis rates when exercise is performed in the fed state. The application of a

single-leg exercise protocol allowed differentiation between the impact of exercise and the systemic changes in substrate availability and hormonal responses due to the intake of food. In addition, we determined the impact of exercise on the phosphorylation status of several signaling proteins known to play a key role in the regulation of muscle protein synthesis at two time points after exercise and/or food intake. Resistance exercise substantially increases muscle protein synthesis rates, accompanied by an elevated phosphorylation state of both 4E-BP1 and S6.

Resistance exercise has been reported to stimulate muscle protein synthesis for up to 48 h postexercise (28). Moreover, it has been well established that net protein balance after exercise remains negative until protein and/or amino acids are administered (3,28). Numerous studies have demonstrated that the ingestion of intact protein (21,22,33,34) or amino acids (12,16,25,30,35) further stimulate muscle protein synthesis during postexercise recovery. However, these studies compared the interventions to the basal, that is, fasted, resting condition. Few previous studies have compared the additive effect of exercise + nutrition versus nutrition only. In these studies, resistance exercise was carried out in the fasted state followed by the infusion of amino acids (3) or the continuous administration of small amounts of a mixed macronutrient drink (27,31,32). Both scenarios are atypical of normal everyday practice, in which exercise activities are commonly performed after feeding. Furthermore, the pattern of amino acid availability in the blood is different after a bolus ingestion of protein (34) versus continuous infusion or feeding (3,11,27), and this difference might modulate the protein synthetic response (6). The impact of resistance exercise on the protein synthetic rate of muscle already stimulated by food intake has yet to be elucidated.

In the present study, we applied a study design in which untrained males performed an acute bout of unilateral resistance-type exercise 75 min after ingesting a standardized breakfast. Our data demonstrate that mixed muscle protein synthesis rates were significantly higher (~20%) in the Ex versus the NEx muscle (Fig. 3). The observed FSR values are in a range with previously reported protein synthesis rates after amino acid infusion (3) or repeated ingestion of a mixed macronutrient drinks (27,32) during postexercise recovery. We acknowledge that it is not everyday practice to remain fasted during 6 h of postexercise recovery. However, this study design was a prerequisite to allow us to differentiate between the impact of muscle contraction and nutrition on muscle protein synthesis after exercise performed in the fed state. The logical extension to this study design is to add ingestion of protein after exercise, which is a common practice among those participating in resistance exercise programs.

Resistance exercise performed in the fasted state has previously been demonstrated to induce a 100% to 150% increase in FSR compared with a basal (i.e., resting and fasted) situation (28). In the present study, we did not measure basal mixed muscle protein synthetic rate. However, using similar measurement techniques (i.e., tracer incorporation method and choice of IC as precursor) to the present study, previous articles commonly report basal FSR values to be approximately 0.04% to 0.06%·h^{-1} (28,37). Thus, the reported FSR values in the present study would correspond with an approximately 80% to 150% increase from basal muscle protein synthesis rates, values similar to previously published data (28). Therefore, the present study clearly shows that resistance exercise substantially stimulates mixed muscle protein synthesis in conditions where food intake is followed by resistance exercise.

Over the past few years, it has been well established that the mTOR signal-transduction pathway plays a key regulatory role in the control of muscle protein synthesis (18). Downstream targets of mTOR, for example, S6K1 and 4E-BP1, control the rate of translation initiation by regulating the binding of mRNA to the ribosomal subunits (18). Although it has been demonstrated in humans that increases in muscle protein synthesis after either resistance exercise (12,13) or amino acid administration (10,16) are accompanied by the activation of the mTOR signaling pathway, a paucity of data examining the effect of exercise and nutrition on both FSR and molecular signaling currently exists. In our study design, muscle biopsies were collected from Ex and NEx muscle immediately after exercise and after 6 h of postexercise recovery corresponding to 2 and 8 h after food intake (Fig. 1). This approach allowed us to determine potential differences in phosphorylation status of AMPK, 4E-BP1, eEF2, mTOR, S6K1, S6, and eIF4B due to food intake and food intake in combination with exercise at these time points. The present study design is restricted by the number of biopsies that were collected. No biopsies were collected before exercise and/or throughout the different stages of postexercise recovery. Therefore, the present data are not representative of the time course of the changes in mixed muscle protein synthesis and/or skeletal muscle signaling.

It has previously been shown that a single bout of high-intensity resistance-type exercise performed in the fasted state results in skeletal muscle AMPK phosphorylation (13,23). The latter has been associated with a decline in 4E-BP1 phosphorylation and an inhibition of muscle protein synthesis (7,13,23). Therefore, it has been proposed that AMPK plays an important role in the regulation of muscle protein synthesis. However, in the present study, in which exercise was performed in the postprandial state, we did not observe a significant increase in AMPK phosphorylation in the Ex compared with the NEx leg (Fig. 4A). These observations suggest that AMPK is not further activated when resistance-type exercise is performed in a fed state, which would be in line with previously published data showing that feeding reduces AMPK phosphorylation (16). Interestingly, we did observe a significantly lower 4E-BP1 phosphorylation state after exercise in the Ex muscle compared with the NEx leg, although AMPK and mTOR

phosphorylation were similar in muscle tissue from both legs (Fig. 4). The latter is not surprising as this would be in line with previous findings showing that the impact of nutrition is of greater importance in the up-regulation of mTOR phosphorylation when compared with exercise (12,16). Ingestion of essential amino acids has previously been shown to substantially enhance 4E-BP1 phosphorylation in humans (16). Combined with recent observations (20), it seems reasonable to assume that the marked decrease in 4E-BP1 phosphorylation generally observed after exercise (12,23) is ameliorated when exercise is performed in a fed, as opposed to a fasted, state. In the present study, the phosphorylation status of 4E-BP1 was significantly higher (~40%) in the Ex leg after 6 h of postexercise recovery compared with the NEx leg (8 h after meal ingestion; Fig. 4B). The latter tends to be in line with the observation that exercise leads to a more prolonged stimulation of muscle protein synthesis (28) when compared with the protein synthetic response to food intake (5).

The present study shows that phosphorylation of S6K1 at T^{421}/S^{424} tended to be higher after local muscle contraction when compared with the impact of food intake only (Fig. 5). These data confirm that the T^{421}/S^{424} phosphorylation status of S6K1 is predominantly influenced by exercise (23) and is not greatly affected by mere changes in circulating insulin and/or amino acid concentrations (20). Phosphorylation of S6K1 at T^{421}/S^{424} is not necessarily associated with increased activity of the kinase (S6K1). This notion is supported by the observation that exercise only does not increase the phosphorylation status of S6K1 at its activation site (T^{389}) during the early stages of postexercise recovery (14,17,23). Amino acid availability has been suggested to be essential to augment S6K1 phosphorylation at T^{389} after exercise (12,17,20). In the present study, skeletal muscle S6K1 phosphorylation at T^{389} did not differ between the Ex leg at 0 and 6 h of postexercise recovery and the NEx leg (2 and 8 h after food intake; Fig. 5). Despite the lack of significant changes in S6K1 phosphorylation at T^{389}, we did observe greater S6 phosphorylation after cessation of exercise in the active limb (Fig. 5). The latter might be attributed to the inability to sample muscle tissue more frequently, preventing the observation of a peak in S6K1 phosphorylation after exercise and food intake. Alternatively, S6 phosphorylation could also be mediated by another anabolic signaling pathway in addition to the mTOR signaling cascade, that is, MAP-dependant kinase (MAPK) signaling (15,39). Recent studies in rodents (15) and humans (39) have shown activation of MAPK signaling proteins (ERK 1/2, p90RSK, Mnk 1, P38 MAPK, and JNK/SAPK) after resistance-type exercise. However, based on the paucity of currently available data in humans, it is difficult to speculate on the potential role of MAPK signaling proteins in augmenting protein synthesis after muscle contraction.

In the present study, we simultaneously measured protein FSR and the phosphorylation state of key signaling proteins in the mTOR pathway in the Ex and the NEx leg muscle. The classic study by Baar and Esser (1) has shown a strong correlation (r = 0.998) between S6K1 phosphorylation measured after electrical stimulation and subsequent muscle mass gain in rodents. Their data have been used to suggest that the phosphorylation status of key signaling proteins after resistance-type exercise might be applicable as biomarkers for the long-term increase in skeletal muscle mass. In humans, studies examining the effect of exercise and nutrition on both FSR and molecular signaling are quite limited. Recently, it has been demonstrated that increases in muscle protein synthesis rate after either resistance exercise (12,13) or amino acid administration (10,16) are accompanied by the activation of the mTOR signaling pathway. Interestingly, we did not observe any significant correlations between the FSR and the (changes in) phosphorylation state of the different key proteins in the mTOR signaling cascade (data not shown). From a physiological standpoint, lack of association between elevated FSR and anabolic signaling should not necessarily be considered surprising. Biopsies were taken immediately after cessation of exercise and after 6 h of recovery, allowing the assessment of FSR during the entire postexercise recovery period and the determination of anabolic signaling events at these two time points. However, in the present study, we did not aim to assess potential changes in the mTOR signaling cascade that could have occurred during early (2 h) postexercise recovery (12). In addition, it could be speculated that there is a temporal disconnect between phosphorylation of certain signaling proteins and maximal rates of muscle protein synthesis. Furthermore, we acknowledge the potential role of alternate signaling pathways such as MAPK in modulating the synthesis of muscle proteins. Clearly, more *in vivo* human research is warranted to assess the time-dependent changes in both skeletal muscle signaling and FSR. So far, the latter has been restricted by the number of muscle biopsy samples that are being collected, which generally precludes a more detailed insight into the time course of changes in skeletal muscle signaling.

In conclusion, resistance exercise in the fed state substantially increases mixed muscle protein synthesis rate. The greater increase in mixed muscle protein synthesis after muscle contraction is accompanied by an elevated 4E-BP1 and S6 phosphorylation status during postexercise recovery.

R. Koopman was supported with an International Training Fellowship from the Nutricia Research Foundation, The Netherlands (2006-T4).

OCW, KDT, LJCvL, and RK designed the study. OCW and MT organized and carried out the clinical experiments with the assistance of MB. OCW and RK performed the statistical analysis and wrote the manuscript together with KDT and LJCvL. MB provided medical assistance.

We gratefully acknowledge the expert technical assistance of Joan Senden and Jos Stegen and the enthusiastic support of the subjects who volunteered to participate in these experiments. The results of the present study do not constitute endorsement by ACSM. R. Koopman was supported with an International Training Fellowship from the Nutricia Research Foundation, The Netherlands (2006-T4).

REFERENCES

1. Baar K, Esser K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol.* 1999;276(1 Pt 1):C120–7.
2. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest.* 1975;35(7):609–16.
3. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol.* 1997;273(1 Pt 1):E122–9.
4. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol.* 2001;3(11): 1014–9.
5. Bohe J, Low A, Wolfe RR, Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *J Physiol.* 2003; 552(Pt 1):315–24.
6. Bohe J, Low JF, Wolfe RR, Rennie MJ. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol.* 2001;532(Pt 2):575–9.
7. Bolster DR, Crozier SJ, Kimball SR, Jefferson LS. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through downregulated mTOR signaling. *J Biol Chem.* 2002; 277(27):23977–80.
8. Borsheim E, Tipton KD, Wolf SE, Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. *Am J Physiol Endocrinol Metab.* 2002;283(4):E648–57.
9. Chesley A, MacDougall JD, Tarnopolsky MA, Atkinson SA, Smith K. Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol.* 1992;73:1383–88.
10. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 2005;19(3):422–4.
11. Dangin M, Boirie Y, Garcia-Rodenas C, et al. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab.* 2001; 280(2):E340–8.
12. Dreyer HC, Drummond MJ, Pennings B, et al. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *Am J Physiol Endocrinol Metab.* 2008;294(2):E392–400.
13. Dreyer HC, Fujita S, Cadenas JG, Chinkes DL, Volpi E, Rasmussen BB. Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol.* 2006;576(Pt 2):613–24.
14. Eliasson J, Elfegoun T, Nilsson J, Kohnke R, Ekblom B, Blomstrand E. Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply. *Am J Physiol Endocrinol Metab.* 2006;291(6):E1197–205.
15. Fluckey JD, Dupont-Versteegden EE, Knox M, Gaddy D, Tesch PA, Peterson CA. Insulin facilitation of muscle protein synthesis following resistance exercise in hindlimb-suspended rats is independent of a rapamycin-sensitive pathway. *Am J Physiol Endocrinol Metab.* 2004;287(6):E1070–5.
16. Fujita S, Dreyer HC, Drummond MJ, et al. Nutrient signalling in the regulation of human muscle protein synthesis. *J Physiol.* 2007;582(Pt 2):813–23.
17. Karlsson HK, Nilsson PA, Nilsson J, Chibalin AV, Zierath JR, Blomstrand E. Branched-chain amino acids increase p70S6 kinase phosphorylation in human skeletal muscle after resistance exercise. *Am J Physiol Endocrinol Metab.* 2004;287(1):E1–E7.
18. Kimball SR, Farrell PA, Jefferson LS. Invited review: role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *J Appl Physiol.* 2002; 93(3):1168–80.
19. Koopman R, Beelen M, Stellingwerff T, et al. Co-ingestion of carbohydrate with protein does not further augment post-exercise muscle protein synthesis. *Am J Physiol Endocrinol Metab.* 2007; 293(3):E833–42.
20. Koopman R, Pennings B, Zorenc AH, van Loon LJ. Protein ingestion further augments S6K1 phosphorylation in skeletal muscle following resistance type exercise in males. *J Nutr.* 2007;137:1836–42.
21. Koopman R, Verdijk LB, Manders RJF, et al. Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *Am J Clin Nutr.* 2006;84(3):623–32.
22. Koopman R, Wagenmakers AJ, Manders RJ, et al. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab.* 2005;288(4): E645–53.
23. Koopman R, Zorenc AH, Gransier RJ, Cameron-Smith D, van Loon LJ. The increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers. *Am J Physiol Endocrinol Metab.* 2006;290(6): E1245–E52.
24. Kubica N, Bolster DR, Farrell PA, Kimball SR, Jefferson LS. Resistance exercise increases muscle protein synthesis and translation of eukaryotic initiation factor 2Bepsilon mRNA in a mammalian target of rapamycin-dependent manner. *J Biol Chem* 2005;280(9):7570–80.
25. Miller SL, Tipton KD, Chinkes DL, Wolf SE, Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. *Med Sci Sports Exerc.* 2003;35(3): 449–55.
26. Nader GA, Esser KA. Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. *J Appl Physiol.* 2001;90(5):1936–42.
27. Phillips SM, Parise G, Roy BD, Tipton KD, Wolfe RR, Tamopolsky MA. Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state. *Can J Physiol Pharmacol.* 2002;80(11):1045–53.
28. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol.* 1997;273(1 Pt 1):E99–107.
29. Phillips SM, Tipton KD, Ferrando AA, Wolfe RR. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am J Physiol.* 1999;276(1 Pt 1):E118–24.
30. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol.* 2000;88(2):386–92.
31. Tang JE, Manolakos JJ, Kujbida GW, Lysecki PJ, Moore DR, Phillips SM. Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men. *Appl Physiol Nutr Metab.* 2007;32(6): 1132–8.
32. Tang JE, Perco JG, Moore DR, Wilkinson SB, Phillips SM. Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol.* 2008;294(1):R172–8.
33. Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *Am J Physiol Endocrinol Metab.* 2007;292(1):E71–6.
34. Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, Wolfe RR. Ingestion of casein and whey proteins result in muscle

anabolism after resistance exercise. *Med Sci Sports Exerc.* 2004;36(12):2073–81.
35. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol.* 1999;276(4 Pt1): E628–34.
36. van Eijk HM, Rooyakkers DR, Deutz NE. Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2–3 microns Spherisorb ODS II column. *J Chromatogr.* 1993;620(1):143–8.
37. Volpi E, Sheffield-Moore M, Rasmussen BB, Wolfe RR. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA.* 2001;286(10):1206–12.
38. Welle S, Bhatt K, Thornton CA. Stimulation of myofibrillar synthesis by exercise is mediated by more efficient translation of mRNA. *J Appl Physiol.* 1999;86(4):1220–5.
39. Williamson D, Gallagher P, Harber M, Hollon C, Trappe S. Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. *J Physiol.* 2003:547(3):977–87.