Effects of Elevated Circulating Hormones on Resistance Exercise-Induced Akt Signaling

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ABSTRACT

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Purpose: Hormones and muscle contraction alter protein kinase B (Akt) signaling via distinct mechanisms. Therefore, the purpose of this study was to determine whether physiologically elevated circulating hormones modulate resistance exercise (RE)-induced signaling of Akt and its downstream targets. We hypothesized that elevated circulating hormones would potentiate the signaling response. **Methods:** Seven healthy men (mean ± SD age, 27 ± 4 yr; body mass, 79.1 ± 13.6 kg; body fat, 16% ± 7%) performed two identical lower-body RE protocols (five sets of five maximal repetitions of knee extensions) in a randomized order and separated by 1–3 wk: one protocol was preceded by rest [low-circulating hormonal concentration (LHC) trial], and the other was preceded by a bout of high-volume upper-body RE using short rest periods designed to elicit a large increase in circulating hormones [high-circulating hormonal concentration (HHC) trial]. **Results:** The HHC trial invoked significantly ($P \leq 0.05$) greater growth hormone (GH) and cortisol concentrations compared with the LHC trial. There were minimal differences between trials in insulin and insulin-like growth factor-I (IGF-I) concentrations. Contrary to our hypothesis, 70-kDa ribosomal protein S6 kinase (p70 S6K) threonine (Thr) 389 phosphorylation within the *vastus lateralis* was attenuated at 180 min post-RE during the HHC trial. RE did not affect Akt or glycogen synthase kinase-3β (GSK-3β) phosphorylation nor were there differences between trials. Immediately post-RE, eukaryotic initiation factor (eIF) 4E binding protein-1 (4E-BP1) phosphorylation declined, and adenosine monophosphate-activated protein kinase (AMPK) phosphorylation increased; however, there were no differences between trials in these variables. **Conclusion:** p70 S6K Thr 389 phosphorylation was attenuated during the HHC trial despite dramatically greater (>2.5-fold) circulating GH concentrations; this was potentially due to cortisol-induced inhibition of p70 S6K Thr 389 phosphorylation. **Key Words:** ENDOCRINE, mTOR, PKB, MUSCLE SIGNALING

BASIC SCIENCES

Muscle overload produced by contraction, stretch, and/or external resistance potently stimulates muscle growth (2,4,40). This favorable adaptation results from protein accretion within existing muscle fibers, promoting fiber hypertrophy. Various models of muscle overload demonstrate that enhanced translational efficiency mediates the initial increase in protein synthesis after muscle overload (8,39,40) and that the protein kinase B (Akt) pathway signals this response (4). Akt signaling phosphorylates downstream targets, including translation initiation factors and ribosomal proteins, to increase translational efficiency and protein synthesis (21). In addition to promoting hypertrophy, Akt signaling also prevents atrophy. Mice that have been genetically modified to express a constitutively active form of Akt maintain and even increase muscle mass despite denervation of muscle (4). Therefore, Akt signaling represents an intriguing target for research aimed at promoting muscle growth and attenuating the muscle atrophy characteristic of aging, unloading, and various diseases.

Phosphorylation at serine (Ser) 473 allows Akt to subsequently phosphorylate two important downstream targets to ultimately increase translational efficiency: the mammalian target of rapamycin (mTOR) and glycogen synthase kinase-3β (GSK-3β). Akt phosphorylates mTOR on Ser 2448, which increases the kinase activity of mTOR (30). Subsequently, mTOR phosphorylates the 70-kDa ribosomal protein S6 kinase (p70 S6K) at threonine (Thr) 389 and hyperphosphorylates eukaryotic initiation factor (eIF) 4E

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binding protein 1 (4E-BP1). Phosphorylation at Thr 389 activates p70 S6K, causing it to subsequently phosphorylate the S6 subunit of the 40S ribosomal protein. Phosphorylation of S6 increases the translation of mRNAs encoding ribosomal proteins and translational factors. 4E-BP1 normally binds to and inhibits eIF4E; however, hyperphosphorylation of 4E-BP1 by mTOR causes it to release from eIF4E. Subsequently, eIF4E binds mRNA and congregates with eIF4A (an RNA helicase) and eIF4G (a scaffolding protein) to form the heterotrimeric translation initiation complex eIF4F. Akt also phosphorylates GSK-3β at Ser 9; this relieves the inhibition of eIF2B by GSK-3β. Subsequently, eIF2B chaperones methionyl-mRNA to the 40S ribosomal subunit for translation initiation (for review of Akt-mTOR signaling, see Ref. (21)). Altogether, Akt signaling increases protein synthesis by activating stimulators (i.e., p70 S6K, eIF2B) and by repressing inhibitors (i.e., 4E-BP1, GSK-3β) of translational efficiency.

Stimuli of the Akt pathway include hormones and muscle contraction. Insulin (30), insulin-like growth factor-I (IGF-I) (31), and growth hormone (GH) (7) bind to their respective membrane-bound receptors and subsequently activate phosphatidylinositol-3 kinase (PI-3K), an upstream stimulus for Akt Ser 473 phosphorylation. Alternately, cortisol binds to and converts the intracellular glucocorticoid receptor to a transcription factor capable of translocating to the nucleus and associating with DNA to regulate glucocorticoid-specific gene expression. The activated glucocorticoid receptor then induces expression of unidentified protein(s) that suppress signaling downstream of Akt (as indicated by inhibited p70 S6K and 4E-BP1 phosphorylation) (34). Unlike hormones, muscle contraction stimulates mTOR independently of PI-3K or Akt (19), instead relying on phospholipase D-induced phosphatidic acid production (18). At rest, α-actinin in the z-band of the sarcomere associates with and inhibits phospholipase D (27). However, as proposed by Hornberger et al. (18), phospholipase D dissociates from α-actinin during mechanical deformation of muscle fibers; this relieves the inhibition of phospholipase D by α-actinin and subsequently promotes phosphatidic acid production and subsequent mTOR activation. Altogether, these *in vitro* studies indicate that hormones and muscle contraction activate Akt signaling via distinct mechanisms.

Although *in vitro* studies capably isolate the independent influences of muscle contraction and hormones, these variables are inextricably linked during *in vivo* resistance exercise (RE). Muscle contractions performed during RE can simultaneously invoke considerable hormonal responses, including increases in circulating GH (24), circulating and muscle IGF-I (3,24), and circulating cortisol (25). GH and IGF-I strongly stimulate Akt signaling, whereas cortisol opposes it. Because hormones and muscle contraction influence muscle signaling via distinct mechanisms (intra-/extracellular receptors and phosphatidic acid, respectively), it is conceivable that circulating hormones interact with muscle contractions to alter RE-induced signaling of Akt and its downstream targets (Fig. 1 presents our theoretical paradigm). However, this potential interrelationship remains to be examined during *in vivo* RE. Therefore, the purpose of this study was to determine the relative importance of circulating hormones for modulating (either stimulating or inhibiting) RE-induced signaling of Akt and its downstream targets. We hypothesized that high concentrations of circulating hormones would potentiate RE-induced phosphorylation of Akt and its downstream targets because RE invokes large increases in circulating GH, a known stimulus of translational efficiency.

METHODS

Experimental Approach

Subjects performed two identical lower-body RE protocols in a randomized order: One protocol was preceded by rest, and the other was preceded by a bout of high-volume upper-body RE using short rest periods. Given the physiological and metabolic demands, one trial (lower-body exercise only) resulted in low-circulating hormonal concentrations (LHC) and the other trial (lower-body and upper-body exercise) resulted in high-circulating hormonal

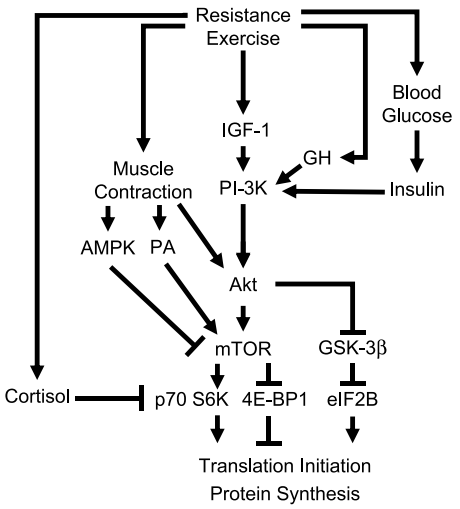

FIGURE 1—Theoretical paradigm demonstrating the numerous potential influences of RE on Akt signaling. Muscle contractions performed during RE incite a considerable hormonal response, including IGF-I, GH, and cortisol. Additionally, RE mildly increases blood glucose, which promotes insulin release. IGF-I, GH, and insulin stimulate Akt signaling, whereas cortisol inhibits p70 S6K and 4E-BP1 phosphorylation. Muscle contraction *per se* stimulates mTOR via phosphatidic acid. However, muscle contraction can also stimulate AMPK activity, which inhibits mTOR activity. Arrows signify a stimulatory effect; blocked lines indicate an inhibitory effect. Abbreviations: Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; eIF2B, eukaryotic initiation factor 2B; GH, growth hormone; GSK-3β, glycogen synthase kinase-3β; IGF-I, insulin-like growth factor-I; mTOR, mammalian target of rapamycin; PA, phosphatidic acid; PI-3K, phosphatidylinositol-3 kinase; p70 S6K, 70-kDa ribosomal protein S6 kinase; 4E-BP1, eukaryotic initiation factor 4E binding protein-1.

concentrations (HHC). Figure 2 displays the study protocol. Experimental trials were separated by 1–3 wk.

Subjects

Seven physically active men participated in this study (mean ± SD age, 27 ± 4 yr; height, 177 ± 5 cm; body mass, 79.1 ± 13.6 kg; body fat, 16% ± 7%). Because resistance-trained men display blunted mTOR signaling response compared with untrained men (9), individuals who participated in a resistance training program within the previous 3 months were excluded from the study. Subjects received a verbal explanation of the study procedures and the associated risks. Subsequently, subjects provided written informed consent. The University of Connecticut Institutional Review Board for use of human subjects approved all procedures.

Procedures

Study controls. Subjects recorded all dietary intake during the 2 d preceding each experimental trial. During this time, subjects refrained from exercise and ingestion of alcohol and stimulants (including caffeine). Diet records from the first trial were photocopied and returned to subjects before the second trial with instructions to match previous intake as closely as possible.

All experimental trials were performed in the morning after a 12-h overnight fast (except for water). Time of day was standardized (±1 h) to avoid confounding influences of diurnal hormonal variations. Additionally, subjects were instructed to drink 0.5 L of water the night before and 0.5 L of water the morning of the experimental trials to ensure adequate hydration. This study control was based on previous research from our laboratory showing that hydration status can influence hormonal responses to RE (unpublished). Adequate hydration (urine specific gravity ≤ 1.020) was confirmed before the experimental protocol via urine refractometry.

Familiarization and one-repetition maximum (1-RM) testing. For the initial visit, subjects reported to the laboratory for familiarization with the leg extension, bench press, bench row, and shoulder press exercises. Additionally, height and body mass were measured, and skinfold thicknesses were determined via calipers to estimate percent body fat. After familiarization, subjects were tested for 1-RM strength in the leg extension, bench press, bench row, and shoulder press exercises. Briefly, subjects performed a warm-up on a cycle ergometer followed by light stretching. Then, subjects performed 8–10 repetitions at 50% of estimated 1-RM, followed by another set of 3–5 repetitions at 85% of 1-RM. Three to four maximal trials separated by 2–3 min of rest were used to determine individual 1-RM for each RE. One-repetition maximum strength for the leg extension, bench press, bench row, and should press was 112 ± 19 kg, 64 ± 10 kg, 65 ± 9 kg, and 50 ± 9 kg, respectively.

Lower-body exercise protocol. During the LHC and HHC trials, subjects performed a lower-body exercise protocol that consisted of five sets of five RM (90–95% of 1-RM) bilateral, concentric–eccentric knee extensions with 3 min of rest between sets. This heavy-load knee extension exercise protocol involved low total work and long rest periods, thus stimulating a minimal hormonal response. Before the knee extension exercise, investigators individualized the equipment settings for each subject such that the machine's fulcrum was aligned with the knee joint, the leg pad was positioned at 5 cm above the ankle, and the starting position of the exercise was set at 90° of knee flexion. These settings were determined during familiarization, recorded for each subject, and repeated for all trials. The resistance used and the number of repetitions performed during the first experimental trial were recorded and replicated exactly during the second experimental trial. Because the lower-body exercise stimulus during the two experimental trials was identical, any difference between trials in muscle signaling was due to differences in systemic (e.g., hormonal) factors.

Upper-body exercise protocol. During the HHC trial, subjects performed an upper-body exercise protocol that consisted of four sets of 10 RM each of the bench press, bench row, and seated shoulder press exercises (in that order). For each exercise, the initial load was 80% of individual 1-RM. If muscle failure occurred during a set, then spotters provided assistance until the subject completed the remaining repetitions and resistance was reduced

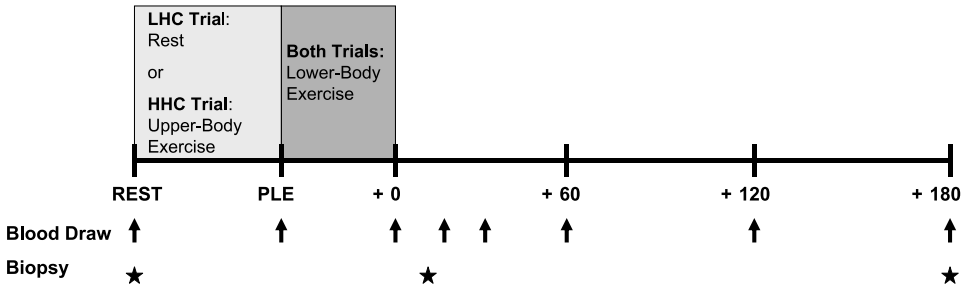

FIGURE 2—Study protocol. Experimental trials were randomized and separated by 1–3 wk. Abbreviations: HHC, high-circulating hormonal concentrations; LHC, low-circulating hormonal concentrations; PLE, before lower-body exercise. Numerical values refer to number of minutes postexercise.

for subsequent sets. In all cases, 2 min of rest separated sets and exercises. This high-volume, short rest period protocol maximized the hormonal response to exercise (24).

The bench press exercise began with the subject lying supine on a bench, holding a barbell at full elbow extension. On command, the subject lowered the bar to mid-chest and then lifted the bar back to full elbow extension. Subjects began the bench row exercise by lying prone on a bench, holding a barbell at full elbow extension. On command, the subject raised the bar to the bottom of the bench and then lowered the bar back to full elbow extension. The shoulder press began with the subject in a seated position, holding a barbell at full elbow extension. On command, the subject lowered the bar past his chin and then raised the bar back to full elbow extension. For each of the upper-body exercises, subjects assumed a position (either seated or lying on a bench) that minimized activation of the leg musculature; this equated the lower-body exercise stimulus between trials.

Blood draws. Before commencing experimental trials, a trained phlebotomist inserted an indwelling Teflon cannula into a superficial forearm vein of the subject. The cannula was kept patent with a 10% heparin–saline solution. During the LHC and the HHC trials, venous blood samples were obtained at rest (REST), before the lower-body exercise protocol (PLE), immediately post-RE (+0), and at 15-min (+15), 30-min (+30), 60-min (+60), 120-min (+120), and 180-min (+180) post-RE. Before each blood draw, 3 mL of blood was drawn and discarded to avoid inadvertent dilution of the blood sample. For each blood draw, 10 mL of blood was collected and transferred into appropriate tubes for obtaining serum and plasma and then centrifuged at 1500*g* for 15 min at 4°C. Resulting serum and plasma was aliquoted and stored at −80°C until subsequent analyses.

Muscle biopsies. During each experimental trial, small (30–50 mg) muscle samples were obtained from the *vastus lateralis* using the percutaneous needle biopsy technique with suction (14). Briefly, the skin over the muscle was gently shaved with a razor, disinfected using Betadine, and anesthetized using 2% lidocaine. A small (1 cm) incision was made through the skin and muscle fascia with a scalpel and then a sterile biopsy needle was introduced into the muscle to obtain the muscle sample. Muscle samples were cleared of excess blood and connective tissue, flash frozen in liquid nitrogen, and stored at −80°C until subsequent analyses. During a given experimental trial, each biopsy was obtained from the same leg but through a separate incision. Each incision was separated by 3 cm to avoid potential confounding influences of immune or inflammatory responses to the biopsy procedure on Akt signaling. The contralateral leg was biopsied during the subsequent experimental trial, again using three separate incisions.

During each trial, biopsies were obtained at REST, at exactly 10 min post-RE (+10), and at 180 min post-RE (+180). Post-RE biopsy time points were chosen because previous research demonstrated that 1) RE-induced Akt and 4E-BP1 phosphorylation peaks at 10 min postexercise (5); 2) RE-induced adenosine monophosphate-activated protein kinase (AMPK) activity peaks between 0 and 60 min postexercise (12); and 3) RE-induced p70 S6K phosphorylation lags 30–180 min after the cessation of exercise (9,12,20,22). Therefore, the +10 and +180 biopsies were chosen to capture peak responses of Akt/4E-BP1/AMPK and p70 S6K, respectively.

Biochemical analyses. Serum samples were analyzed in duplicate for insulin, IGF-I, GH, and cortisol via commercially available enzyme-linked immunosorbent assays (ELISA) (Diagnostic Systems Laboratory, Webster, TX). Coefficient of variation for these assays was 5.5%, 1.5%, 7.3%, and 2.8%, respectively. All samples were analyzed in one run to avoid interassay variation. Additionally, plasma samples were analyzed in duplicate for lactate and glucose concentrations (YSI Stat Plus, Yellow Springs, OH). Coefficient of variation for lactate and glucose analyses was <3% for all samples. Serum and plasma samples were thawed only once before analysis.

Muscle samples were analyzed in triplicate for Akt (total and phosphorylated at Ser 473), GSK-3β (total and phosphorylated at Ser 9), p70 S6K (total and phosphorylated at Thr 389), 4E-BP1 (phosphorylated at Thr 46), and AMPK (phosphorylated at Thr 172) using commercially available phospho-ELISAs (BioSource, Carlsbad, CA) that were validated for sensitivity (ELISA sensitivity was approximately twofold greater than Western Blot analysis), intra- and interassay precision (coefficient of variation is <10.0% for all analytes), recovery (>90% for all analytes), and specificity (antibodies used to detect phosphopeptides were specific for the particular phosphorylation site; the responses of total and phosphopeptides to a given stimulus were similar when measured by ELISA as compared with Western blot analysis).

Before analysis, each phospho-ELISA was optimized for buffer composition and total protein added per well. For the Akt and the GSK-3β phospho-ELISAs, samples were homogenized using NP40 Cell Lysis Buffer (BioSource), containing 50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4, and 1% Nonidet P40. For p70 S6K, 4E-BP1, and AMPK phospho-ELISAs, samples were homogenized using Tissue Extraction Reagent I (BioSource), containing 50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 1 mM NaF, 2 mM Na_3VO_4, 20 mM $Na_4P_2O_7$, 0.02% NaN_3, and a proprietary detergent. Immediately after homogenization, samples were analyzed for total protein concentration using a detergent-compatible, reagent-compatible protein assay (BioRad, Hercules, CA). For all phospho-ELISAs, 30 μg of total protein was added per well. Phospho-Akt, phospho-GSK-3β, and phospho-p70 S6K values ($U \cdot mL^{-1}$) were normalized for total Akt, total GSK-3β, and total p70 S6K ($ng \cdot mL^{-1}$), respectively, and for total protein added per well (and therefore reported

as U·ng^{-1}). Phospho-4E-BP1 and phospho-AMPK values were normalized for total protein added per well only (and therefore reported as U·mL^{-1}). Coefficient of variation for these assays was 7.4% for total Akt, 6.0% for phospho-Akt, 20.3% for total GSK-3β, 8.0% for total GSK-3β, 17.5% for total p70 S6K, 16.3% for phospho-p70 S6K, 7.2% for phospho-4E-BP1, and 14.8% for phospho-AMPK. Although each assay was optimized, during the experimental analyses the optical absorbance of the unknowns was located on the low-end of the standard curve. Therefore, small absolute differences in absorbance among triplicates resulted in exaggerated coefficients of variation for some analytes (i.e., total GSK, total and phospho-p70 S6K, phospho-AMPK). All samples were analyzed in one run to avoid interassay variation. Muscle samples were thawed only once before analysis.

Statistical Analyses

Sample size was calculated using the data of Coffey et al. (9), as their RE stimulus and subject population closely resembled those of the current study. Using those data and examining a two-tailed hypothesis at a power of 0.80, the number of subjects required to demonstrate a significant difference in p70 S6K phosphorylation (a key dependent variable in this study) at $P = 0.05$ was calculated to be 3. Measures of central tendency and variation were calculated for all variables; obvious outliers (>2 SD) were removed from the data set. Data are presented as mean ± SD unless otherwise stated. Area under the time curve (AUC) was calculated for all hormonal variables using standard trapezoidal methods. Data were analyzed using a trial × time repeated-measures ANOVA. In the event of a significant F score, the Fisher LSD *post hoc* test was used to determine pairwise differences. The criterion for statistical significance was set *a priori* at $P \leq 0.05$.

RESULTS

Hormonal responses to RE. The HHC protocol significantly ($P \leq 0.05$) elevated GH concentrations above REST values at PLE, +0, +15, and +30 (Fig. 3A). Moreover, HHC values were greater than LHC values at PLE, +0, +15, +30, and +60. The GH AUC was 2.5-fold greater during the HHC trial than the LHC trial; this response was consistent among individual subjects (Fig. 3A insert). The LHC protocol elevated GH concentrations above REST only at +0. These results confirmed that activating a greater

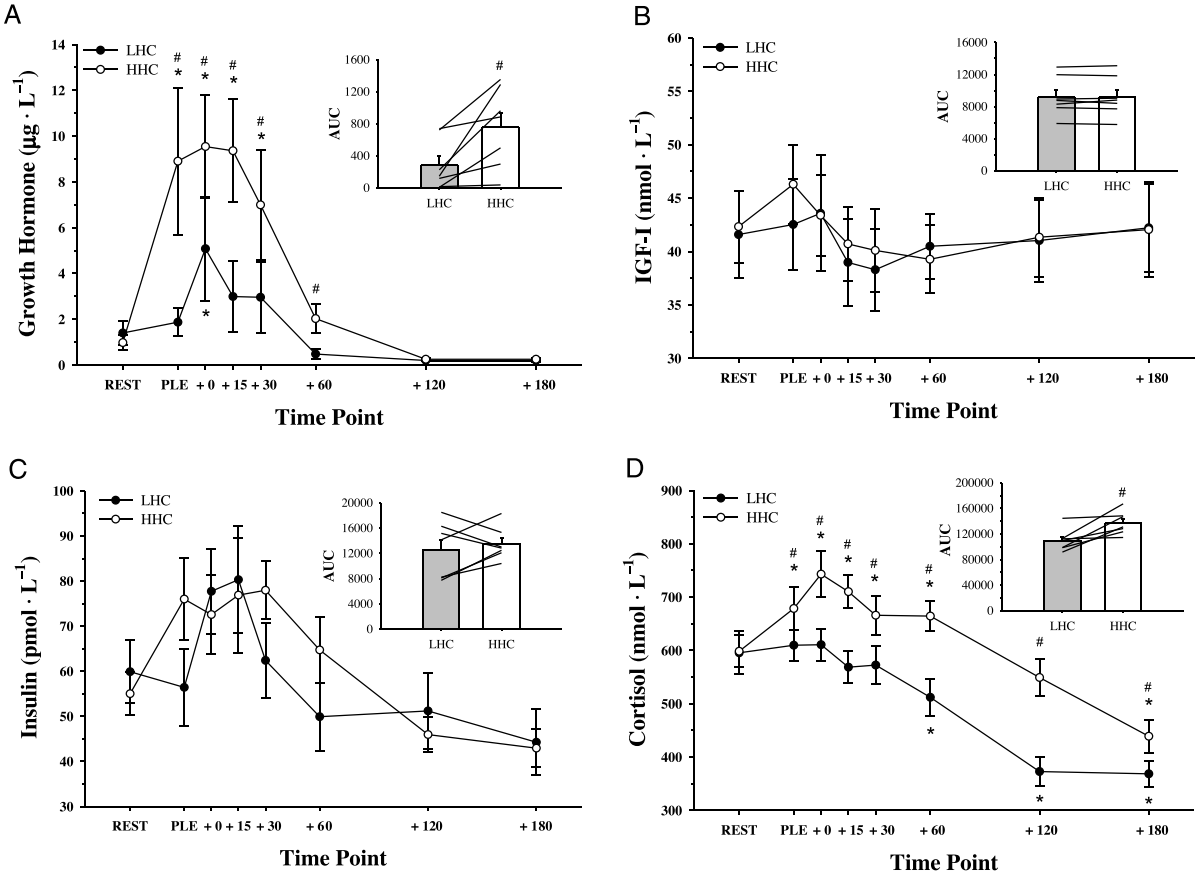

FIGURE 3—Responses (mean ± SE) of GH (Panel A), IGF-I (Panel B), insulin (Panel C), and cortisol (Panel D) to the different RE protocols. AUC is presented in panel inserts; lines indicate individual responses. * Significantly ($P \leq 0.05$) different than corresponding REST value; # significantly ($P \leq 0.05$) different than corresponding LHC value.

TABLE 1. Plasma lactate and glucose responses (mean ± SD) to RE.

	Lactate (mmol·L^{-1})		Glucose (mmol·L^{-1})	
	LHC	HHC	LHC	HHC
REST	1.46 ± 0.31	1.33 ± 0.28	5.57 ± 0.40	5.37 ± 0.43
PLE	1.49 ± 0.46	12.03 ± 1.88*,†	5.57 ± 0.74	6.24 ± 1.04*,†
+0	5.78 ± 1.09*	10.63 ± 1.39*,†	5.71 ± 0.50	6.42 ± 0.89*,†
+15	3.62 ± 0.84*	7.54 ± 1.20*†	6.08 ± 0.96	6.23 ± 1.00*
+30	2.11 ± 0.65	5.09 ± 1.29*,†	5.55 ± 0.47	5.88 ± 1.04*,†
+60	1.26 ± 0.22	2.73 ± 0.82*,†	5.34 ± 0.45	5.17 ± 0.49
+120	1.00 ± 0.15	1.37 ± 0.30	5.39 ± 0.40	5.35 ± 0.43
+180	1.23 ± 0.48	1.15 ± 0.13	5.26 ± 0.45	5.30 ± 0.43

* Significantly ($P \leq 0.05$) different than corresponding REST value.
† Significantly ($P \leq 0.05$) different than corresponding LHC value.

amount of muscle mass during RE produces a greater GH response than protocols recruiting smaller muscle masses (15).

The influence of RE while in a fasted state on IGF-I (23,24) and insulin (25) responses is equivocal. In the present investigation, RE did not alter circulating IGF-I values at any time point nor were there any differences between trials (Fig. 3B). Individual IGF-I AUC values were similar between trials (Fig. 3B insert). Alternately, RE increased circulating insulin (main effect of time), with concentrations peaking at +15 and reaching a nadir at +180 (Fig. 3C). However, there was no time × trial interaction ($P = 0.087$) and no difference between trials in insulin AUC. Individual insulin responses to the two exercise trials were variable (Fig. 3C insert).

The HHC protocol increased cortisol concentrations above REST values at PLE, +0, +15, +30, and +60 (Fig. 3D). The HHC trial stimulated greater cortisol concentrations compared with the LHC trial at all postexercise time points and in the cortisol AUC; this was consistent among individuals (Fig. 3D insert). Cortisol concentrations typically display a distinct diurnal variation: concentrations peak in the early morning and fall throughout the day. This diurnal variation explained why cortisol values fell below REST values at +180 during the HHC trial and at +60, +120, and +180 during the LHC trial.

In addition to hormones, we also measured plasma concentrations of lactate (a marker of metabolic stress) and glucose (the primary insulin secretogogue). The HHC protocol increased lactate concentrations above REST values at PLE, +0, +15, +30, and +60 (Table 1). The LHC trial elevated lactate only at +0 and +15. Lactate values during the HHC trial were significantly greater than LHC values at PLE, +0, +15, +30, +60, and for the lactate AUC. The HHC trial increased glucose concentrations above REST values at PLE, +0, +15, and +30 (Table 1). The LHC trial caused no significant changes in glucose concentrations.

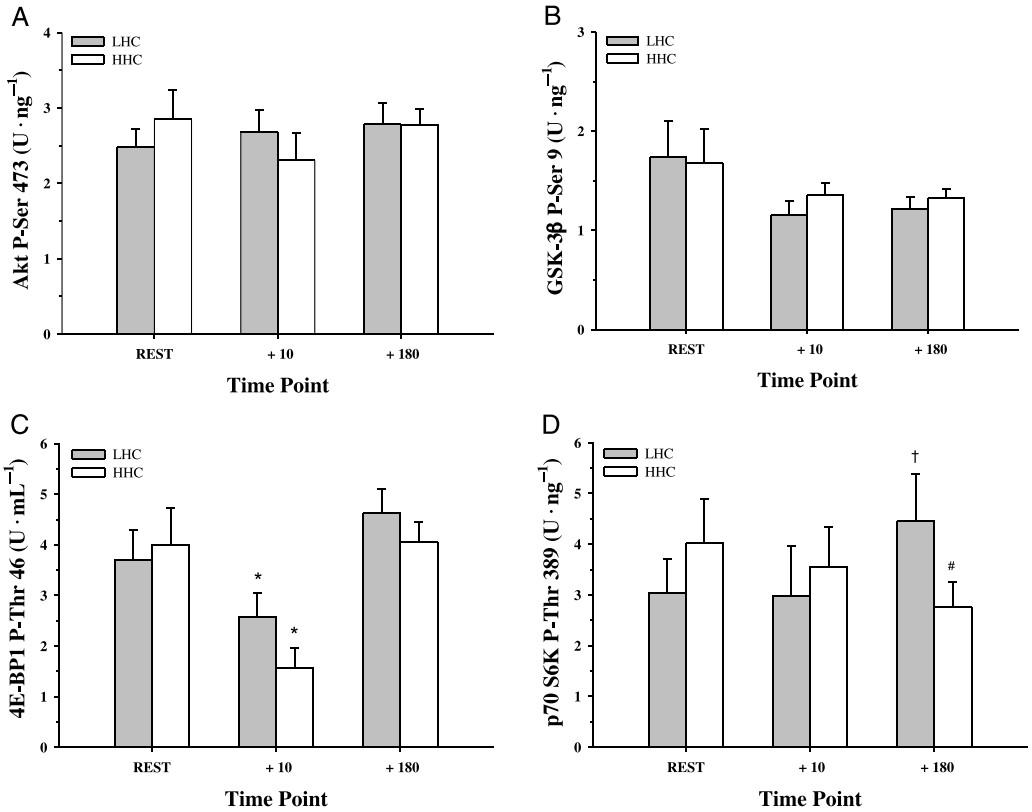

FIGURE 4—Responses (mean ± SE) of Akt phosphorylation at Ser 473 (Akt P-Ser 473; Panel A), glycogen synthase kinase-3β phosphorylation at Ser 9 (GSK-3β P-Ser 9; Panel B); 70-kDa ribosomal protein S6 kinase phosphorylation at Thr 389 (p70 S6K P-Thr 389; Panel C), and eukaryotic initiation factor 4E binding protein-1 phosphorylation at Thr 46 (4E-BP1 P-Thr 46; panel D) to different RE protocols. *Significantly ($P \leq 0.05$) different than corresponding REST value; # significantly ($P \leq 0.05$) different than corresponding LHC value; † trend ($P = 0.095$) for difference compared with corresponding REST value.

Glucose values during the HHC trial were significantly greater than LHC values at PLE, +0, and +30; however, there was no difference between trials in the glucose AUC.

Muscle signaling responses to RE. The influence of RE on the phosphorylation status of Akt and its downstream targets was enzyme specific. We found no change in Akt Ser 473 (Fig. 4A) or GSK-3β Ser 9 (Fig. 4B) phosphorylation in response to RE nor were there any differences between trials. A preponderance of individuals showed no discernible change in Akt phosphorylation from REST to IP; variable responses existed from IP to +180 (data not shown). For GSK-3β, interindividual variation existed at REST; however, the responses to RE during both trials were homogeneous (data not shown). Although RE decreased 4E-BP1 Thr 46 phosphorylation at +0, there were no differences between trials (Fig. 4C). Individual responses in 4E-BP1 phosphorylation were similar (data not shown).

The LHC trial tended (P = 0.095) to increase p70 S6K Thr 389 phosphorylation above REST at +180 (Fig. 4D). This agrees with previous human studies showing that RE-induced increases in p70 S6K phosphorylation lag 30–180 min after the cessation of exercise (9,12,20,22). Contrary to our hypothesis, though, p70 S6K phosphorylation at +180 was attenuated during the HHC trial compared with the LHC trial. Figure 5 displays individual p70 S6K phosphorylation values at the +180 time point and individual cortisol AUC values for both trials. Five of the seven subjects had lower p70 S6K phosphorylation at +180 during the HHC trial. Although only a nonsignificant correlation existed (r = −0.37), a relationship appeared to exist between p70 S6K phosphorylation at +180 and cortisol AUC (e.g., subjects 1, 2, 4, and 7 had much greater cortisol AUC during the HHC trial, which coincided with attenuated p70 S6K phosphorylation, and subject 6 had no discernible difference between trials in cortisol or in p70 S6K phosphorylation) (Fig. 5).

Previous investigations have shown that RE increases AMPK activity, an inhibitor of Akt signaling (5). In the present study, RE increased AMPK Thr 172 phosphorylation immediately postexercise; however, there were no differences between trials (Fig. 6).

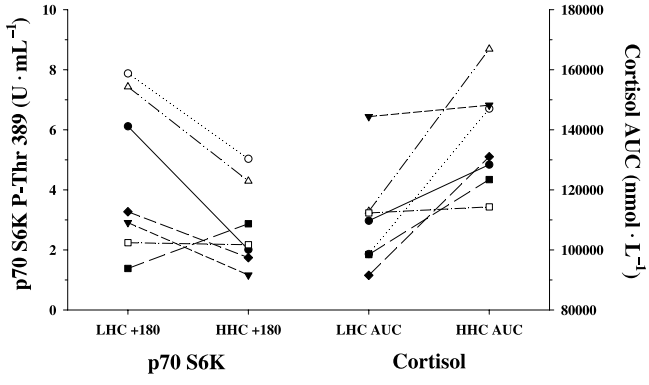

FIGURE 5—Individual responses of 70-kDa ribosomal protein S6 kinase phosphorylation at Thr 389 (p70 S6K P-Thr 389) at 180 min post-RE (left side) and cortisol AUC (right side). Like symbols represent responses for the same individual.

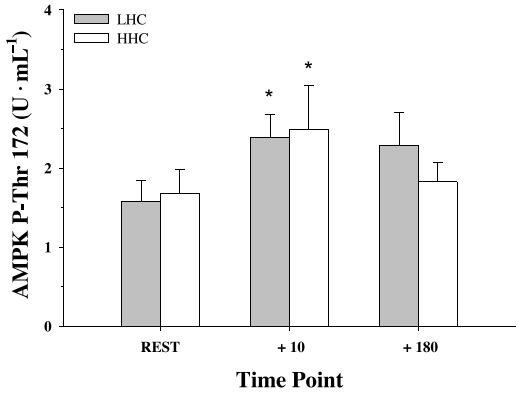

FIGURE 6—Responses (mean ± SE) of AMPK phosphorylation at Thr 172 (AMPK P-Thr 172) to RE. * Significantly ($P \leq 0.05$) different than corresponding REST value.

DISCUSSION

Previous research demonstrated that GH (a hormone potently stimulated by RE (24)) activates enzymes downstream of Akt and subsequently enhances translational efficiency and protein synthesis (7). Therefore, we hypothesized that physiologically elevated circulating hormones would enhance signaling of Akt and its downstream targets after an acute bout of RE. Contrary to our hypothesis, though, physiologically elevated circulating hormones did not potentiate phosphorylation of Akt or its downstream targets. We instead found that p70 S6K Thr 389 phosphorylation was attenuated during the HHC trial despite dramatically greater circulating GH concentrations.

To ascertain the potential mechanism(s) of attenuated p70 S6K signaling during the HHC trial, we measured two effective inhibitors of Akt signaling: muscle AMPK phosphorylation and circulating cortisol concentrations. AMPK is postulated to be the cellular "energy sensor" because high adenosine monophosphate and low glycogen concentrations (markers of low cellular energy) activate AMPK (5). In response to this decrease in energy, AMPK promotes energy-releasing pathways (e.g., glucose and fatty acid oxidation) and inhibits energy-consuming pathways (e.g., protein synthesis) during times of energy shortage (e.g., exercise). AMPK reduces protein synthesis via inhibition of mTOR and its downstream targets (5). In the present study, AMPK phosphorylation at Thr 172 (a critical site for AMPK activity) was similar during the LHC and HHC trials (Fig. 6); this was not surprising when considering that we measured AMPK phosphorylation in the *vastus lateralis* and that the lower-body RE protocol was identical during the two experimental trials. Therefore, differences in AMPK activity cannot explain attenuated p70 S6K signaling during the HHC trial.

On the other hand, a considerable difference in circulating cortisol concentrations (Fig. 3D) reasonably explains attenuated p70 S6K phosphorylation during the HHC trial. A series of studies by Shah et al. (35–37) demonstrated that glucocorticoids inhibit particular aspects of Akt signaling. Specifically, dexamethasone (a synthetic glucocorticoid) inhibited p70 S6K phosphorylation at Thr 389 but did not inhibit Akt Ser 473 phosphorylation or eIF2B activity (35–37). These results (35–37) are remarkably similar to those of the present study: we found that greater cortisol concentrations during the HHC trial were concomitant with attenuated p70 S6K phosphorylation at Thr 389, whereas Akt Ser 473 phosphorylation and GSK-3β Ser 9 phosphorylation (which regulates eIF2B activity) were unaffected. Shah et al. (37) also demonstrated that glucocorticoids significantly inhibit p70 S6K phosphorylation within 2 h of administration, providing further support that RE-induced cortisol elevations plausibly inhibited p70 S6K phosphorylation at 3 h postexercise in the present investigation. Therefore, we propose that circulating cortisol concentrations determined the differences between the HHC and the LHC trial in p70 S6K Thr 389 phosphorylation.

Although glucocorticoids inhibit both 4E-BP1 and p70 S6K phosphorylation (36), important differences exist in glucocorticoid-induced inhibition of these enzymes. Whereas glucocorticoid-induced inhibition of 4E-BP1 phosphorylation was rescued by IGF-I, p70 S6K phosphorylation was not (36). Apparently, glucocorticoid-induced expression of unidentified protein(s) promoted autoinhibition of p70 S6K, which reduced the efficiency with which p70 S6K is phosphorylated at Thr 389 (34). This unique difference between p70 S6K and 4E-BP1 might explain why we found that only p70 S6K Thr 389 phosphorylation was attenuated during the HHC trial. Because IGF-I and GH activate Akt signaling through similar mechanisms (i.e., via PI-3K) (29), it is conceivable that greater GH concentrations during the HHC trial rescued the cortisol-induced inhibition of 4E-BP1 phosphorylation (similar IGF-I rescuing dexamethasone-induced inhibition of 4E-BP1 phosphorylation (36)).

Previous research clearly demonstrated that physiologically elevated circulating hormones potentiate physical adaptations after RE training (15). Paradoxically, however, we found that physiologically elevated circulating hormones attenuated p70 S6K Thr 389 phosphorylation, indicating that protein synthesis was likely attenuated as well (2). The fasted status of the subjects in the present study potentially explains these discrepancies. In the context of the present study, the importance of nutrient intake for Akt signaling is threefold. First, Karlsson et al. (20) showed that RE increased p70 S6K Thr 389 phosphorylation in humans only if branched chain amino acids were ingested. Similarly, we found that p70 S6K Thr 389 phosphorylation did not increase after RE in fasted humans [although there was a trend ($P = 0.095$) for increased p70 S6K phosphorylation at +180 in the LHC trial], further supporting the critical role of nutrients for RE-induced Akt signaling. Second, GH injection increased muscle protein synthesis, translational efficiency, association of eIF4E with eIF4G, and p70 S6K phosphorylation in fed but not fasted pigs (7). This might explain why the observed increases in GH during the HHC trial failed to increase p70 S6K phosphorylation in fasted humans after RE. Third, one study showed that glucose ingestion blunted the RE-induced cortisol response and subsequently potentiated the adaptations to RE training (38). Therefore, future research should delineate the interaction between nutrient intake and circulating hormones on RE-induced Akt signaling; extended muscle sampling (i.e., beyond the initial 3 h after RE) should also be considered.

RE invokes a host of physiological effects ranging from systems-level responses (e.g., endocrine, immune) to gene-level responses (e.g., transcription, translation). Thus, it is impossible to state with certainty the exact mechanism(s) for reduced p70 S6K phosphorylation during the HHC trial. Ironically, though, this limitation (i.e., use of an *in vivo* model) is also the primary strength of this investigation. The ultimate goal of many *in vitro* models of contraction-induced changes in skeletal muscle signaling is to eventually develop physiologic, pharmacologic, and/or genetic interventions designed to promote hypertrophy and/or offset atrophy in humans. Initial research using human models sets a foundation for future research into RE-induced muscle signaling and muscle growth. Specifically, the present results contribute to a growing body of literature demonstrating that in humans, exercise-induced signaling of Akt and/or its downstream targets is modulated by ingestion of branched-chain amino acids (20), glycogen availability (10), muscle action (11,13), muscle fiber type examined (22), and, potentially, circulating hormonal concentrations (present results). These factors must be considered when designing future *in vivo* studies of exercise-induced muscle signaling.

Important differences between *in vitro* and *in vivo* models of muscle contraction potentially explain discrepancies between our findings and previous studies and provide further support for the use of human models to describe the role of RE in modulating Akt signaling. Electrically stimulated muscle activity increases Akt (1,16,17,26,33), GSK-3β (1), p70 S6K (1,16,19,26), and 4E-BP1 (1) phosphorylation *in vitro*. Alternately, *in vivo*, muscle contraction equivocally alters these variables. Exercise does not change (9,13,28) or increase (6,10–12,32) Akt phosphorylation in humans and intact rats. We found no change in GSK-3β phosphorylation; however, to our knowledge, we are the first to investigate GSK-3β phosphorylation after RE in humans. Mediating variables such as nutrient availability and muscle action likely explain the differences between *in vitro* and *in vivo* studies in p70 S6K phosphorylation after muscle contraction. However, it is difficult to appropriately compare *in vitro* and *in vivo* models in nutrient availability. Finally, although muscle contraction increases 4E-BP1 phosphorylation *in vitro* (1), we and others (12,22) found that RE decreases 4E-BP1 phosphorylation in humans. The mediating factor(s) for this discrepancy is not readily

apparent; however, increased AMPK activity potentially explains this response in humans (13). Much more research is clearly necessary to fully elucidate the influence of muscle contraction on Akt signaling and its downstream targets in humans. However, the importance of such research cannot be understated because Akt signaling represents a potentially critical target for promoting muscle growth and attenuating the muscle atrophy characteristic of aging, unloading, and various diseases.

In conclusion, we investigated the role of physiologically elevated circulating hormones in modulating RE-induced Akt signaling in fasted men. We hypothesized that high concentrations of circulating hormones would potentiate Akt signaling because RE invokes large increases in circulating GH, a known stimulus of translational efficiency. Contrary to our hypothesis, we found that REs eliciting large hormonal responses attenuated p70 S6K Thr 389 phosphorylation. We proposed that elevated cortisol concentrations inhibited p70 S6K phosphorylation during the high-hormonal trial. Finally, the lack of dietary intake might best explain the somewhat contradictory finding that p70 S6K phosphorylation was attenuated despite dramatically greater GH concentrations.

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