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Low muscle glycogen concentration does not suppress the anabolic response to resistance exercise

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Camera DM, West DW, Burd NA, Phillips SM, Garnham AP, Hawley JA, Coffey VG. Low muscle glycogen concentration does not suppress the anabolic response to resistance exercise. *J Appl Physiol* 113: 206–214, 2012. First published May 24, 2012; doi:10.1152/japplphysiol.00395.2012.—We determined the effect of muscle glycogen concentration and postexercise nutrition on anabolic signaling and rates of myofibrillar protein synthesis after resistance exercise (REX). Sixteen young, healthy men matched for age, body mass, peak oxygen uptake ($\dot{V}O_{2peak}$) and strength (one repetition maximum; 1RM) were randomly assigned to either a nutrient or placebo group. After 48 h diet and exercise control, subjects undertook a glycogen-depletion protocol consisting of one-leg cycling to fatigue (LOW), whereas the other leg rested (NORM). The next morning following an overnight fast, a primed, constant infusion of L-[*ring*-$^{13}C_6$] phenylalanine was commenced and subjects completed 8 sets of 5 unilateral leg press repetitions at 80% 1RM. Immediately after REX and 2 h later, subjects consumed a 500 ml bolus of a protein/CHO (20 g whey + 40 g maltodextrin) or placebo beverage. Muscle biopsies from the vastus lateralis of both legs were taken at rest and 1 and 4 h after REX. Muscle glycogen concentration was higher in the NORM than LOW at all time points in both nutrient and placebo groups ($P < 0.05$). Postexercise Akt-p70S6K-rpS6 phosphorylation increased in both groups with no differences between legs ($P < 0.05$). mTOR^{Ser2448} phosphorylation in placebo increased 1 h after exercise in NORM ($P < 0.05$), whereas mTOR increased ~4-fold in LOW ($P < 0.01$) and ~11 fold in NORM with nutrient ($P < 0.01$; different between legs $P < 0.05$). Post-exercise rates of MPS were not different between NORM and LOW in nutrient (0.070 ± 0.022 vs. 0.068 ± 0.018 %/h) or placebo (0.045 ± 0.021 vs. 0.049 ± 0.017 %/h). We conclude that commencing high-intensity REX with low muscle glycogen availability does not compromise the anabolic signal and subsequent rates of MPS, at least during the early (4 h) postexercise recovery period.

skeletal muscle; muscle protein synthesis

SKELETAL MUSCLE GLYCOGEN CONCENTRATION exerts numerous regulatory effects on cell metabolism in response to contraction (23). Indeed, commencing endurance-based exercise with low muscle glycogen availability has been shown to increase the maximal activities of several oxidative enzymes in skeletal muscle that promote endurance adaptation (21, 46). Although the anabolic effects of resistance-based exercise on skeletal muscle are well established (9), little is known regarding the effects of altered muscle glycogen concentration availability on the acute protein synthetic response to resistance exercise and whether the summation of these responses may enhance or attenuate training-induced adaptation.

The complex regulatory process of protein synthesis after muscle contraction and/or protein ingestion includes activation of the Akt-mTOR-S6K signaling pathway to initiate translation (16, 38). Numerous studies have addressed the signaling responses to resistance exercise under a variety of nutritional states (i.e., fasted/fed) (10, 43). However, the effects of muscle glycogen availability have yet to be clearly elucidated. Work by Creer and colleagues (11) showed an attenuation in Akt phosphorylation during recovery when subjects commenced a bout of moderate-intensity resistance exercise with low (~175 mmol/kg dry wt) vs. high (~600 mmol/kg dry wt) muscle glycogen. Furthermore, contraction-induced translational signaling may be suppressed when energy-sensing AMPK activity is increased (1, 42). Wojtaszeski and coworkers (44) have observed elevated resting and exercise-induced AMPK activity when muscle glycogen levels were low (~160 mmol/kg dry wt) compared with high (~910 mmol/kg dry wt). Moreover, work from our laboratory also previously demonstrated low muscle glycogen concentration has the capacity to alter basal transcription levels of select metabolic and myogenic genes (8). Thus the increased metabolic perturbation when exercising in a low glycogen state might be expected to inhibit the anabolic response to resistance exercise.

It is well accepted that protein intake following resistance exercise is critical for optimizing many of the training-induced adaptations in skeletal muscle (25). Ingestion of high-quality protein has been shown to enhance translation initiation signaling and maximally stimulate muscle protein synthesis rates after resistance exercise (30, 35). Carbohydrate (CHO) ingestion provides substrate for muscle glycogen resynthesis, but has no additive effect on rates of muscle protein synthesis after resistance exercise (29). The capacity for protein-carbohydrate coingestion in the early postexercise period to rescue any putative attenuation of muscle protein synthesis when resistance exercise is performed with low glycogen availability has not been investigated. Accordingly, the primary aims of this study were to determine the effect of *1*) decreased muscle glycogen concentration on the acute anabolic response after resistance exercise performed in the fasted state; and *2*) the effect of protein/CHO supplementation on muscle cell signaling and myofibrillar protein synthesis rates (19) following exercise commenced with low muscle glycogen. We hypothesized that low muscle glycogen concentration would suppress the muscle anabolic response to resistance exercise but that nutrient provision in the early recovery period after exercise

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would restore muscle anabolism to a state that may promote hypertrophy.

METHODS

Subjects

Sixteen healthy physically fit male subjects who had been participating in regular concurrent resistance and endurance training (~3×/wk; >1 yr) volunteered for this study. Subjects were randomly assigned to either a nutrient [n = 8, age 22.9 ± 2.6 yr, body mass 80.6 ± 8.8 kg, peak oxygen uptake ($\dot{V}O_{2peak}$) 49.8 ± 5.4 ml·kg^{-1}·min^{-1}, unilateral leg press one repetition maximum (1RM) ~141.7 ± 4.6 kg] or placebo group [age 22.5 ± 4.4 yr, body mass 78.2 ± 4.7 kg, $\dot{V}O_{2peak}$ 47.2 ± 6.9 ml·kg^{-1}·min^{-1}, unilateral leg press 1RM ~141.8 ± 0.8 kg; values are mean ± SD]. The experimental procedures and possible risks associated with the study were explained to each subject, who all gave written informed consent before participation. The study was approved by the Human Research Ethics Committee of RMIT University.

Preliminary Testing

$\dot{V}O_{2peak}$. Peak oxygen uptake was determined during an incremental test to volitional fatigue on a Lode cycle ergometer (Groningen, The Netherlands). The protocol has been described in detail previously (24). In brief, subjects commenced cycling at a workload equivalent to 2 W/kg for 150 s. Thereafter, the workload was increased by 25 W every 150 s until volitional fatigue (defined as the inability to maintain a cadence >70 revolutions/min). Throughout the test, which typically lasted 12–14 min, subjects breathed through a mouthpiece attached to a metabolic cart (Parvomedics, Sandy, UT) to determine oxygen consumption.

Maximal strength. One repetition of maximal dynamic strength (1RM) for each leg was determined on a plate loaded 45° leg press machine (CalGym, Caloundra, Australia). Subjects completed the test with feet placed at the bottom edge of the foot plate and range of motion was 90° knee flexion/extension.

Familiarization to exercise training sessions. To familiarize subjects to one-legged cycling (described subsequently), each subject completed three familiarization sessions before the experimental trial. These sessions consisted of 2 × 10 min bouts of one-legged cycling, with a 2 min recovery period between repetitions. The power output was gradually increased so that by the final session subjects were performing one-legged cycling at ~75% of their two-legged $\dot{V}O_{2peak}$ (37).

Diet/exercise control. Before the exercise depletion session (described subsequently), subjects were instructed to refrain from exercise training and vigorous physical activity and alcohol and caffeine consumption for a minimum of 48 h. A CHO-based diet (~9 g/kg body mass) was consumed 36 h before the one-legged exercise depletion session. All food and drinks were supplied to subjects prepackaged with a food checklist to record their daily intake.

One-legged glycogen depletion protocol. Subjects began a one-legged cycling depletion session at a power output that elicited ~75% of two-legged $\dot{V}O_{2peak}$. The duration of each work bout was 10 min, with 2 min rest between work bouts. Subjects maintained this work-to-rest ratio until volitional fatigue. At this time, power output was decreased by 10 W and subjects cycled at this (lower) work rate with the same work-to-rest ratio until fatigue. After a 10-min rest, subjects then completed 90-s one-leg maximal sprints on a Repco RE7100 Ergo (Altona North, Australia), with 60 s of recovery between work bouts. This protocol was continued until volitional fatigue, defined as the inability to maintain 70 revolutions/min. To further lower whole body glycogen stores and minimize glycogen resynthesis in the LOW leg, subjects completed 30 min of arm cranking on a Monark Rehab Trainer 881E (Vansbro, Sweden). Following the exercise depletion session, subjects were fed a low CHO (~1 g/kg body mass) evening meal.

Experimental Testing Session

On the morning of an experimental trial, subjects reported to the laboratory after a ~10-h overnight fast. After resting in the supine position for ~15 min, catheters were inserted into the antecubital vein of each arm and a baseline blood sample (~3 ml) was taken (Fig. 1). A primed constant intravenous infusion (prime: 2 μmol/kg; infusion: 0.05 μmol·kg^{-1}·min^{-1}) of L-[*ring*-$^{13}C_6$] phenylalanine (Cambridge Isotopes Laboratories) was then administered. Under local anesthesia (2–3 ml of 1% Xylocaine) a resting biopsy from the vastus lateralis of both legs was obtained 1.5 h after commencement of the tracer infusion using a 5-mm Bergstrom needle modified with suction. At this time, two separate sites on each leg (~5 cm distal from each other) were prepared for subsequent biopsies. Subjects then completed a standardized unilateral warm-up (1 × 5 repetitions at 50% and 60% 1RM) on a leg-press machine before the resistance exercise testing protocol was commenced. Resistance exercise consisted of eight sets of five repetitions at ~80% of 1RM for each leg. The glycogen-depleted leg (LOW) began the protocol, with ~60 s rest before the rested normal leg (NORM) completed the same set. Each set was separated by a 3-min recovery period during which the subject remained seated on the machine. The training volume and intensity and recovery interval were selected to provide sufficient anabolic/hypertrophy stimulus and minimize metabolic perturbation and has been used previously (7, 10). If the LOW leg could not complete the

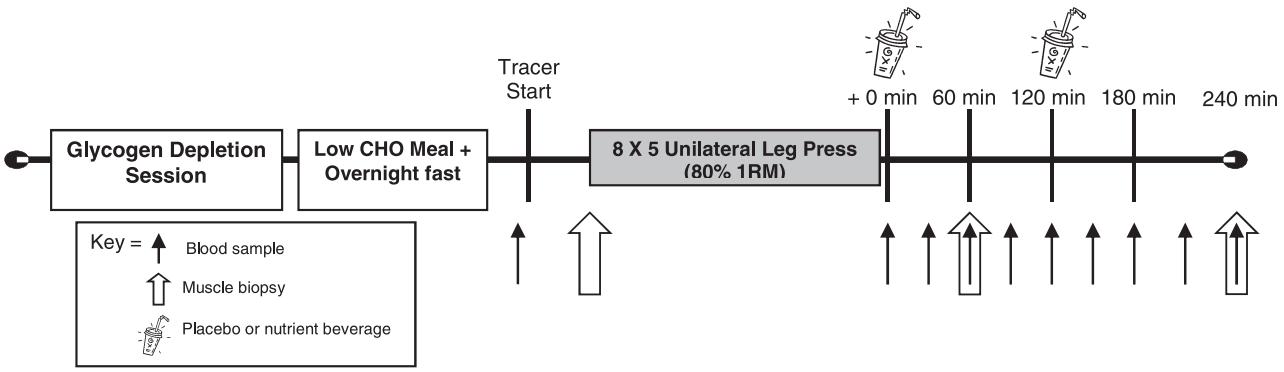

Fig. 1. Schematic representation of the experimental trial. Subjects reported to the laboratory the evening before an experimental trial and performed a 1-legged glycogen-depletion protocol to fatigue before consuming a low carbohydrate (CHO) meal. After an overnight fast, a constant infusion of L-[*ring*-$^{13}C_6$] phenylalanine was commenced, and subjects completed 8 sets of 5 unilateral leg press repetitions at 80% one repetition maximum (1RM). Immediately after resistance exercise (REX) and 2 h later, subjects consumed a 500-ml bolus of a protein/CHO beverage (20 g whey + 40 g maltodextrin) or placebo. Muscle biopsies from both legs (vastus lateralis) were taken at rest and at 1 and 4 h after REX.

repetitions, the NORM leg replicated the number of repetitions to ensure the exercise was work matched and the weight was decreased 5% for subsequent sets. Immediately after the cessation of exercise and 2 h postexercise, subjects ingested a 500 ml placebo (water, artificial sweetener) or protein-CHO beverage (20 g whey protein, 40 g maltodextrin). The nutrient beverage was enriched with a small amount of tracer (to 6.5% of L-[*ring*-$^{13}C_6$] phenylalanine) according to the measured phenylalanine content of the beverage. Subjects rested throughout a 240-min recovery period, and additional muscle biopsies were taken 60 and 240 min postexercise and the samples were stored at −80°C until analysis. Blood samples were collected in EDTA tubes at regular intervals during the postexercise recovery period.

Analytical Procedures

Blood glucose and plasma insulin concentration. Whole blood samples were immediately analyzed for glucose concentration using an automated glucose analyzer (YSI 2300, Yellow Springs, OH). Blood samples were then centrifuged at 1,000 *g* at 4°C for 15 min, with aliquots of plasma frozen in liquid N_2 and stored at −80°C. Plasma insulin concentration was measured using a radioimmunoassay kit according to the manufacturer's protocol (Linco Research).

Plasma amino acids and enrichment. Plasma amino acid concentrations were determined by HPLC from a modified protocol (34). Briefly, 100 μl of plasma was mixed with 500 μl of ice cold 0.6 M PCA and centrifuged at 15,000 rpm for 2 min at 4°C. The PCA was neutralized with 250 μl of 1.25 M potassium bicarbonate ($KHCO_3$), and the reaction was allowed to proceed on ice for 10 min. Samples were then centrifuged at 15,000 rpm for 2 min at 4°C, and the supernatant was separated from the salt pellet and subsequently derivatized for HPLC analysis. Plasma [*ring*-$^{13}C_6$] phenylalanine enrichments were determined as previously described (17).

Muscle glycogen. A small piece of frozen muscle (~20 mg) was freeze-dried and powdered to determine muscle glycogen concentration (33). Freeze-dried muscle was extracted with 500 μl of 2 M hydrochloric acid (HCl), incubated at 100°C for 2 h, and then neutralized with 1.5 ml of 0.67 M sodium hydroxide for subsequent determination of glycogen concentration via enzymatic analysis with fluorometric detection (Jasco FP-750 spectrofluorometer, Easton, MD) at excitation 365 nm/emission 455 nm.

Western blots. Muscle samples were homogenized in buffer containing 50 mM Tris·HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM DTT, 10 μg/ml trypsin inhibitor, 2 μg/ml aprotinin, 1 mM benzamidine, and 1 mM PMSF. Samples were spun at 18,000 *g* for 30 min at 4°C, and the supernatant was collected for Western blot analysis while the pellet was processed to extract the myofibrillar enriched proteins (described below). After determination of protein concentration using a BCA protein assay (Pierce, Rockford, IL), lysate was resuspended in Laemmli sample buffer, separated by SDS-PAGE, and transferred to polyvinylidine fluoride membranes blocked with 5% nonfat milk, washed with 10 mM Tris·HCl, 100 mM NaCl, and 0.02% Tween 20, and incubated with primary antibody (1:1,000) overnight at 4°C on a shaker. Membranes were incubated with secondary antibody (1:2,000), and proteins were detected via enhanced chemiluminescence (Amersham Biosciences, Buckinghamshire, UK; Pierce Biotechnology) and quantified by densitometry (Chemidoc, BioRad, Gladesville, Australia). All sample (50 μg) time points for each subject were run on the same gel. Polyclonal anti-phospho-Akt^{Ser473} (no.9271), mTOR^{Ser2448} (no. 2971), glycogen synthase (GS)^{Ser641} (no.3891), monoclonal anti-phospho-S6 ribosomal protein$^{Ser235/6}$ (no.4856), AMPKα^{Thr172} (no. 2535), and AS160 (no.2670) were from Cell Signaling Technology (Danvers, MA). Polyclonal anti-phospho-p70S6K^{Thr389} (no. 04–392) was from Millipore (Temecula, CA). When commercially available, positive controls (Cell Signaling Technology) were included confirming the band of interest. Data are expressed relative to α-tubublin (no. 3873, Cell Signaling Technology) in arbitrary units.

RNA Extraction and Quantification

Skeletal muscle tissue RNA extraction was performed using a TRIzol-based kit according to the manufacturer's directions (Invitrogen, Melbourne, Australia, Cat. No. 12183–018A). Briefly, ~15 mg of skeletal muscle tissue was removed from RNAlater-ICE solution and homogenized in TRIzol. After elution through a spin cartridge, extracted RNA was quantified using a QUANT-iT analyzer kit (Invitrogen, Cat. No. Q32852) according to the manufacturer's directions.

Reverse Transcription and Real-Time PCR

First-strand complementary DNA (cDNA) synthesis was performed using commercially available TaqMan Reverse Transcription Reagents (Invitrogen) in a final reaction volume of 20 μl. All RNA samples and control samples were reverse transcribed to cDNA in a single run from the same reverse transcription master mix. Serial dilutions of a template RNA (AMBION; Cat. No. AM7982) were included to ensure efficiency of reverse transcription and for calculation of a standard curve for real-time quantitative polymerase chain reaction (RT-PCR). Quantification of mRNA (in duplicate) was performed on a BioRad iCycler (BioRad). Taqman-FAM-labeled primer/probes for atrogin (Cat. No. Hs01041408) and myostatin (Cat. No. Hs00976237) were used in a final reaction volume of 20 μl. PCR conditions were 2 min at 50 °C for UNG activation, 10 min at 95°C, then 40 cycles of 95°C for 15 s and 60°C for 60 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cat. No. Hs Hs99999905) was used as a housekeeping gene to normalize threshold cycle (CT) values. The relative amounts of mRNAs were calculated using the relative quantification (ΔΔCT) method (31).

Myofibrillar Protein Synthesis

Myofibrillar enriched proteins were isolated according to a modified protocol (35). Briefly, the myofibrillar pellet was solubilized in 0.3 M NaOH, precipitated in 1 M PCA, washed in ethanol, and hydrolyzed overnight with 6 M HCl while being heated to 120°C. Liberated myofibrillar and plasma amino acids (for determination of L-[*ring*-$^{13}C_6$] phenylalanine enrichment) were purified using cation-exchange chromatography (Dowex 50WX8–200 resin; Sigma-Aldrich) and converted to their *N*-acetyl-*n*-propyl ester derivatives for analysis by gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS: Hewlett Packard 6890; IRMS model Delta Plus XP, Thermo Finnagan, Waltham, MA). Intracellular free amino acids (IC) were extracted from a separate piece of wet muscle (~20 mg) with ice-cold 0.6 M PCA. Muscle was homogenized, and the free amino acids in the supernatant were purified by cation-exchange chromatography and converted to their heptafluorobutyric (HFB) derivatives before analysis by GC-MS (models 6890 GC and 5973 MS; Hewlett-Packard, Palo Alto, CA) as previously described (35).

Calculations

The rate of myofibrillar protein synthesis was calculated using the standard precursor-product method: FSR (%/h) = $[(E_{2b} - E_{1b})/(E_{IC} \times t)] \times 100$, where $E_{2b} - E_{1b}$ represents the change bound protein enrichment between two biopsy samples, E_{IC} is the average enrichment of intracellular phenylalanine between the two biopsy samples, and t is the time between two sequential biopsies.

Statistical Analysis

All data were analyzed by two-way ANOVA (two factor: time × glycogen concentration) with Student-Newman-Keuls post hoc analysis. Statistical significance was established when $P < 0.05$ (SigmaS-

tat for windows Version 3.11). Based on our a priori hypothesis that anabolic responses to nutrient administration are significantly elevated compared with placebo as shown previously (6, 15), we chose not to make direct comparisons between nutrient and placebo interventions. Data for Western blotting and mRNA abundance were log-transformed prior to analysis. Log-transformed delta values between data time points were also directly compared and converted to Cohen effect sizes (ES). The default confidence interval was 90% to calculate ES making the same assumptions about sampling distributions that statistical packages use to derive *P* values (26). We interpreted the magnitude of the ES by using conventional threshold values of 0.2 as the smallest effect, 0.5 as a moderate effect, and 0.8 as a large ES (26). All data are expressed as arbitrary unit ± SD.

RESULTS

One-Legged Depletion Session and Muscle Glycogen

The time spent completing the one-legged depletion session at an intensity of ~75% of two-legged Vo_{2peak} was 100 ± 3 min. Subjects also completed an average of 6 ± 2 one-legged maximal effort sprint repetitions.

As intended, the combination of the exercise depletion protocol and dietary manipulation generated divergent muscle glycogen levels that were higher in NORM than LOW at rest for the placebo (382 vs. 176 mmol/kg dry wt; $P < 0.001$) and nutrient groups (383 vs. 184 mmol/kg dry wt; $P < 0.05$; Fig. 2). Glycogen concentration was decreased from rest in the NORM leg in both groups at 1 and 4 h postexercise ($P < 0.05$). However, no significant change from rest was evident in the LOW leg for either group. Muscle glycogen increased between 1 and 4 h postexercise in the LOW leg in the nutrient group (~84 mmol/kg dry wt; $P < 0.01$).

Plasma Insulin, Glucose, and Essential Amino Acids

There were main effects for plasma insulin and glucose concentration in the nutrient but not the placebo group ($P < 0.001$; Fig. 3, *A* and *B*). Peak blood insulin and glucose concentrations occurred at 30 and 150 min postexercise ($P < 0.001$). Plasma essential amino acids (EAA) were elevated about rest 150 min and 180 min ($P < 0.05$) postexercise in the nutrient group only (Fig. 3*C*).

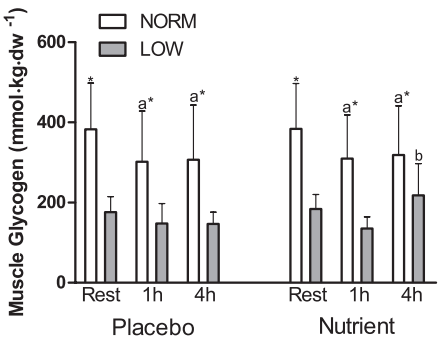

Fig. 2. Muscle glycogen concentration at rest and during 4 h recovery after resistance exercise (8 × 5 leg unilateral leg press at ~80% 1RM) and ingestion of either 500 ml placebo or nutrient beverage immediately post and 2 h postexercise in NORM and LOW glycogen legs. Values are mean ± SD. dw, dry weight. Significantly different ($P < 0.05$) vs. (a) rest, (b) 1 h and (*) between treatments (NORM vs. LOW) at equivalent time point.

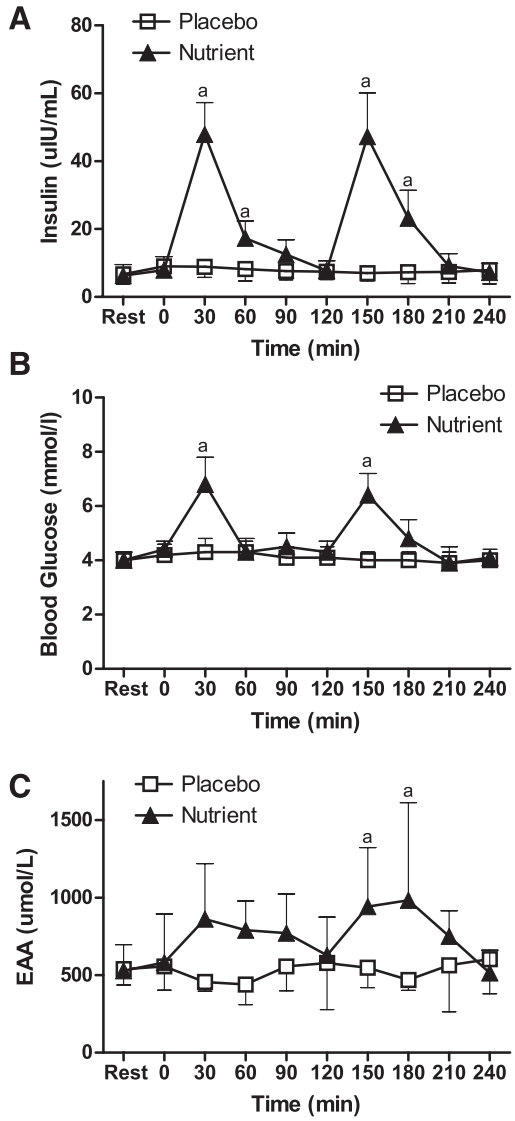

Fig. 3. Plasma insulin (*A*), blood glucose (*B*), and plasma essential amino acid concentration (*C*) at rest and during 240 min recovery following resistance exercise (8 × 5 leg unilateral leg press at ~80% 1RM) and ingestion of either 500 ml placebo or nutrient beverage immediately post and 2 h postexercise. Values are mean ± SD. Significantly different ($P < 0.05$) vs. (a) rest.

Plasma Tracer Enrichments

Plasma L-[*ring* $^{13}C_6$] phenylalanine enrichment at rest and 60, 120, 180, and 240 min postexercise for nutrient and placebo treatments were 0.042, 0.045, 0.055, and 0.049, and 0.058, 0.054, 0.062, 0.057, 0.065, and 0.054 tracer-to-tracee ratio: t/T, respectively. Linear regression analysis indicated that the slopes of the plasma enrichments were not significantly different from zero, demonstrating that isotopic plateau was achieved.

Cell Signaling

Akt-mTOR-p70S6K-rpS6. There were main effects for Akt^{Ser473} phosphorylation for time and glycogen status ($P < 0.05$, Fig. 4A). Resting Akt phosphorylation was higher in LOW than NORM in the placebo group and increased ~2-fold 1 h postexercise in NORM only ($P < 0.05$, ES 0.75) before

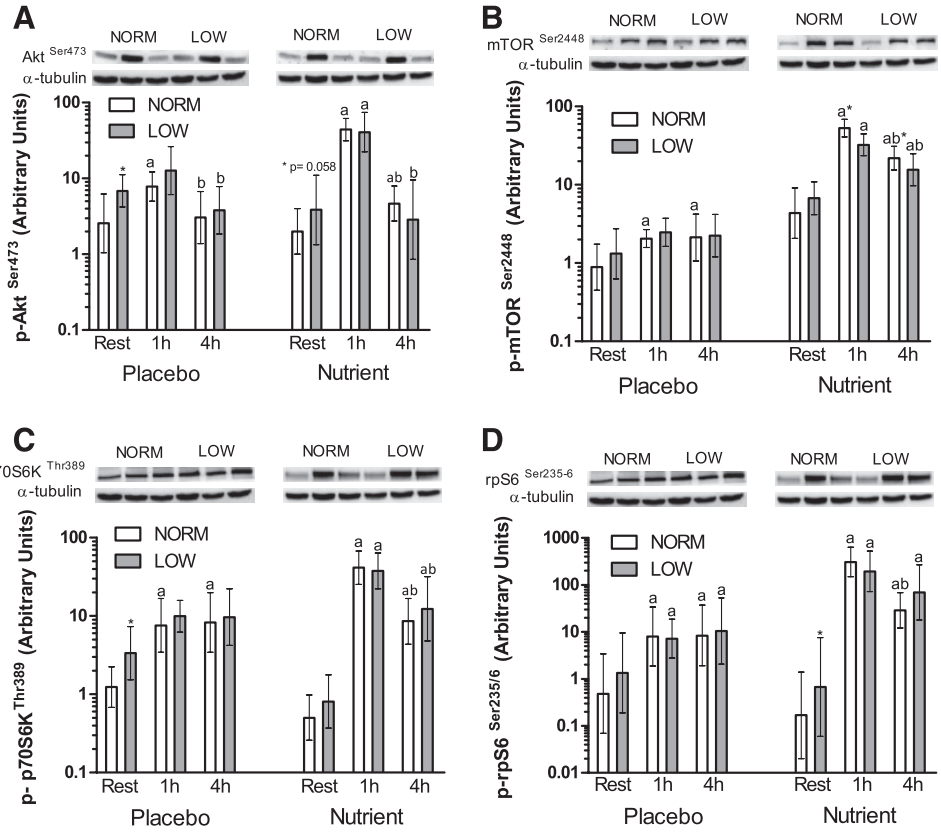

Fig. 4. Akt^{Ser473} (*A*), mammalian target of rapamycin (mTOR)^{Ser2448} (*B*), p70S6K^{Thr389} (*C*), and ribosomal protein S6 (rpS6)$^{Ser235/6}$ (*D*) phosphorylation in skeletal muscle at rest and during 4 h postexercise recovery following resistance exercise (8 × 5 leg unilateral leg press at ~80% one 1RM). Images are representative blots and values are expressed relative to α-tubulin and presented in arbitrary units (mean ± SD, $n = 8$). Significantly different ($P < 0.05$) vs. (a) rest, (b) 1 h, and (*) between treatments (NORM vs. LOW) at equivalent time point.

returning to baseline at 4 h. Phosphorylation at rest was also higher in the LOW compared with the NORM leg ($P = 0.058$) and increased ~10-fold in LOW and ~21-fold in NORM 1 h after resistance exercise in the nutrient group ($P < 0.001$, ES > 1). Akt phosphorylation remained above resting levels following 4 h recovery in the NORM leg only of the nutrient group ($P < 0.05$, ES > 1).

There were main effects for time and glycogen concentration for mTOR^{Ser2448} phosphorylation ($P < 0.05$, Fig. 4*B*). mTOR phosphorylation increased ~1-fold above rest at 1 and 4 h postexercise in the NORM but not the LOW leg in the placebo group ($P < 0.05$, ES ~ 0.5). There was also disparity between legs in the nutrient group that increased in the NORM compared with LOW leg at 1 and 4 h recovery ($P < 0.05$, ES > 1). Phosphorylation in the nutrient group did increase above resting levels ~4-fold and ~1-fold in the LOW leg ($P < 0.01$), an effect that was more pronounced in the NORM leg (~11-fold and ~4-fold, respectively; $P < 0.01$).

p70S6K^{Thr389} phosphorylation was higher at rest in the LOW leg compared with NORM in placebo ($P < 0.05$, Fig. 4*C*). The post–exercise phosphorylation response increased above rest at 1 and 4 h (~5-fold) in NORM but not the LOW leg ($P < 0.01$; ES ~ 1). The comparison of p70S6K phosphorylation between legs at rest in the nutrient group approached significance and was increased above resting levels in both legs at 1 h (LOW: ~45 fold, NORM: ~82 fold, ES > 1; $P < 0.001$) and 4 h (LOW: ~14 fold, NORM: ~16 fold; $P < 0.001$) during recovery from resistance exercise.

There were main effects for rpS6$^{Ser235/6}$ phosphorylation in the nutrient but not placebo group ($P < 0.05$, Fig. 4*D*). There were ~4- and ~6-fold increases in rpS6$^{Ser235/6}$ phosphorylation in the LOW leg with placebo at 1 and 4 h, respectively ($P < 0.05$), and this effect was mirrored in NORM with ~15-fold increases at 1 h ($P < 0.001$, ES 0.9) and 4 h ($P < 0.01$, ES 0.5). Resting rpS6$^{Ser235/6}$ phosphorylation was significantly elevated in the LOW compared with NORM leg ($P < 0.05$) in the nutrient group and increased in both legs 1 h after resistance exercise and remained elevated following 4 h recovery ($P < 0.001$, Fig. 4*D*).

GS-AS160-AMPK. There were significant main effects for GS^{Ser641} phosphorylation for time and glycogen status in placebo and nutrient groups (Fig. 5*A*). GS phosphorylation was markedly higher in NORM than LOW at all time points in the placebo condition ($P < 0.001$). Following resistance exercise phosphorylation decreased ~3- to 4-fold 1 h postexercise in both legs ($P < 0.01$) before increasing at 4 h in the LOW but not NORM leg ($P < 0.01$, ES 0.4). Similarly, phosphorylation was higher at all points in NORM compared with LOW in the nutrient group ($P < 0.05$). There was a decrease from resting levels in the LOW and NORM legs at 1 and 4 h postexercise ($P < 0.05$) but GS phosphorylation only increased between 1 and 4 h in the LOW leg (~5-fold, $P < 0.01$, ES 0.6).

There were main effects for time in placebo and nutrient groups for phospho-AS160 ($P < 0.05$, Fig. 5*B*). AS160 increased in the placebo condition at 1 h (~2-fold $P < 0.01$, ES 0.9) and 4 h (~1-fold $P < 0.05$, ES 0.9) after resistance exercise in the LOW leg only. AS160 phosphorylation in the nutrient group was increased at 1 h recovery in the LOW leg (~4-fold, $P < 0.001$) and both 1 and 4 h postexercise in the NORM leg (~8-fold, $P < 0.001$, ES 0.8; ~1 fold, $P < 0.05$, ES 0.5, respectively).

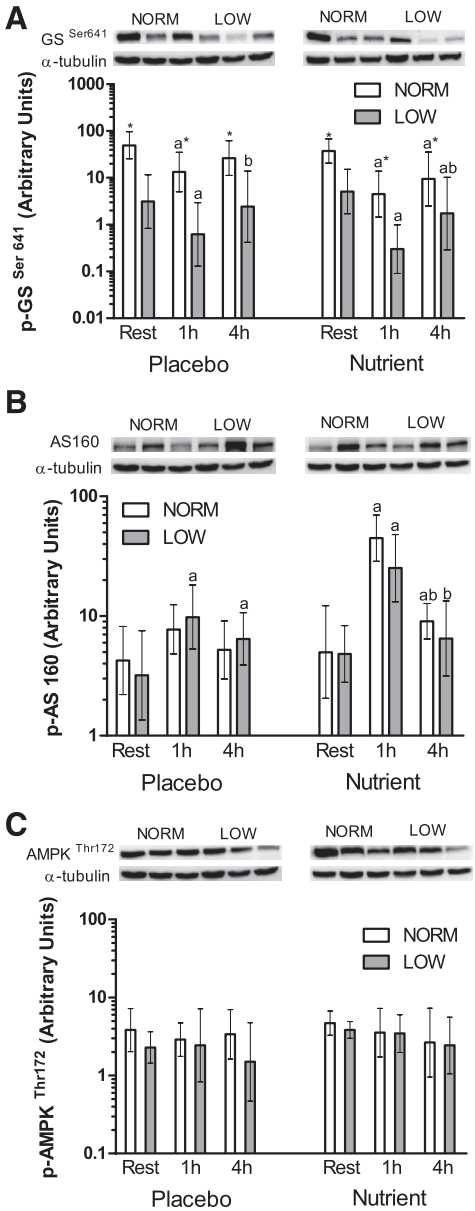

Fig. 5. Glycogen synthase (GS)^{Ser641} (*A*), Akt substrate 160 kDa (AS160) (*B*), and 5′-adenosine monophosphate-activated protein kinase (AMPK)^{Thr172} (*C*) phosphorylation in skeletal muscle at rest and during 4 h postexercise recovery following resistance exercise (8 × 5 leg unilateral leg press at ~80% 1RM). Images are representative blots and values are expressed relative to α-tubulin and presented in arbitrary units (mean ± SD, $n = 8$). Significantly different ($P < 0.05$) vs. (a) rest, (b) 1 h and (*) between treatments (NORM vs. LOW) at equivalent time point.

AMPK^{Thr172} phosphorylation was not different at any time in placebo or nutrient groups (Fig. 5*C*).

Myofibrillar Protein Synthesis

There were no differences in the rates of myofibrillar protein synthesis rates during the 1- to 4-h recovery period between LOW and NORM in placebo (0.049 ± 0.017 vs. 0.045 ± 0.021 %/h) or nutrient (LOW vs. NORM: 0.068 ± 0.018 vs. 0.070 ± 0.022 %/h) conditions (Fig. 6).

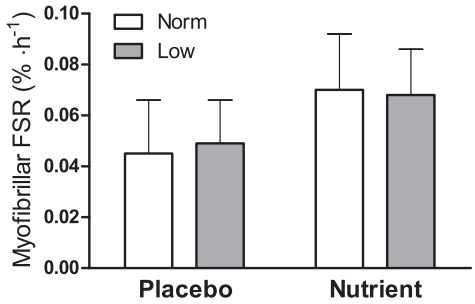

Fig. 6. Myofibrillar protein fractional synthetic rates (FSR) during 4 h of recovery after resistance exercise (8 × 5 leg unilateral leg press at ~80% 1RM) and ingestion of either 500 ml placebo or nutrient beverage immediately post and 2 h postexercise in NORM and LOW glycogen legs. Values are means ± SD.

mRNA expression

Atrogin-myostatin. Atrogin mRNA abundance decreased in the placebo group between rest and 4 h postexercise in the LOW (~1.2-fold; $P < 0.05$) and NORM leg (~1.8-fold; $P < 0.01$) and was also different between 1 and 4 h in NORM (~1.4-fold; ES 0.5, $P < 0.01$) (Fig. 7*A*). Likewise, atrogin mRNA decreased from rest following 4 h recovery in the LOW (~0.5-fold; $P < 0.001$) and NORM leg (~2.6-fold, ES 0.6; $P < 0.001$) in the nutrient group. Atrogin was also different between 1 and 4 h postexercise in the LOW (~0.5-fold, $P < 0.001$) and NORM leg (~2.2-fold, ES 0.25; $P < 0.001$). The mRNA abundance of atrogin was higher in the LOW leg compared with NORM leg at the 4 h postexercise time point in

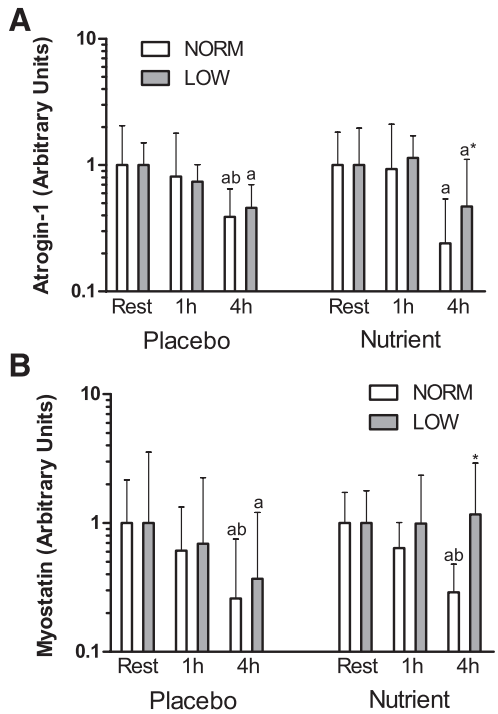

Fig. 7. Atrogin (*A*) and myostatin (*B*) mRNA abundance at rest and during 4 h postexercise recovery following resistance exercise (8 × 5 leg unilateral leg press at ~80% 1RM). Values are expressed relative to GAPDH and presented in arbitrary units (mean ± SD, $n = 8$). Significantly different ($P < 0.05$) vs. (a) rest, (b) 1 h and (*) between treatments (NORM vs. LOW) at equivalent time point.

the nutrient condition ($P < 0.01$). Myostatin mRNA decreased in the placebo group from rest to 4 h in LOW (~1.8-fold; $P < 0.01$) and NORM (~1.4-fold; $P < 0.001$) and between 1 and 4 h recovery in NORM only (~0.8 fold; ES 0.33, $P < 0.01$) (Fig. 7*B*). In the nutrient condition, decreases in myostatin mRNA expression were only observed in the NORM leg that was reduced ~2.4-fold between rest and 1 h (ES ~ 1, $P < 0.01$) and ~1-fold 1–4 h during recovery from resistance exercise (ES 0.8, $P < 0.05$). Myostatin mRNA was higher in the LOW leg compared with NORM leg at 4 h postexercise in the nutrient group ($P < 0.01$).

DISCUSSION

It is generally accepted that skeletal muscle adaptation to repeated bouts of contractile activity are specific to the mode, intensity, and duration of the exercise stimulus (9), but it is unclear how changes in skeletal muscle glycogen availability may modulate nutrient-training interactions to promote or inhibit the adaptive response to resistance exercise. Here we report for the first time that commencing a bout of strenuous resistance exercise with low muscle glycogen concentration has negligible effects on anabolic cell signaling and rates of muscle protein synthesis during the early (4 h) postexercise recovery period. As expected, ingestion of a protein/CHO beverage enhanced the anabolic response to resistance exercise but failed to augment differences between the normal and low glycogen legs.

Exercising in a low glycogen state presents a unique metabolic challenge to skeletal muscle with few studies having investigated the interaction of glycogen content and nutrient provision or their effect on the adaptation response to resistance exercise. Hence the primary novel finding of the present study was that rates of myofibrillar protein synthesis between the NORM and LOW glycogen legs during 1–4 h recovery after resistance exercise were not different (Fig. 6). This finding was unexpected given that acute energy deficit has previously been reported to attenuate rates of mixed muscle protein synthesis by ~19% (36), although the metabolic perturbation with low glycogen in the current study may have had less impact on cell energy status and thus failed to modulate the myofibrillar protein synthetic response to low-volume high-intensity resistance exercise. Nonetheless, our one leg depletion protocol in combination with a low carbohydrate meal was successful in creating divergence in resting muscle glycogen concentration. Muscle glycogen content can be reduced by ~25–40% following a single bout of resistance exercise (7, 39) compared with reductions of ~50% or greater after high-intensity endurance exercise (7, 44). The distinct metabolic demands with endurance exercise may make the adaptation response in mitochondrial and CHO/fat metabolism more sensitive when training with low glycogen, although any benefit to endurance performance has yet to be established (28, 45, 46). In the present study, glycogen availability in the LOW leg may have been sufficient to complete the short periods of contractile activity with long (3 min) recovery between sets without compromising myofibrillar protein synthesis rates during recovery. Moreover, it is possible that greater difference in glycogen availability is necessary to generate differences in metabolic processes that might alter muscle protein synthesis. However, even an endurance exercise bout commenced with low glycogen has only modest effects on muscle protein metabolism (3, 27).

The ingestion of carbohydrate postexercise does not increase muscle protein synthesis in humans per se but we hypothesized carbohydrate coingested with protein may have promoted the anabolic response when muscle glycogen was compromised. In the present study, the nutrient ingestion protocol resulted in divergent plasma glucose, insulin, and amino acid profiles during the 4-h recovery period (Fig. 3). However, we failed to observe an effect of carbohydrate coingestion on anabolic signaling and rates of myofibrillar protein synthesis despite moderate muscle glycogen repletion during the early phase of recovery. Although insulin has been suggested as a potential anabolic hormone that contributes to skeletal muscle accretion (2), recent evidence shows insulin to play only a permissive role in muscle anabolism, at least in young men (29). Despite the availability of carbohydrate for restoring muscle glycogen and the associated increase in plasma insulin levels during recovery in the low glycogen leg, there was no difference in myofibrillar protein synthesis compared with the normal leg. Nonetheless, our results provide further evidence of the well-established capacity for amino acids to augment the muscle protein synthesis response after resistance exercise following an overnight fast.

Another novel finding of our study was that divergent glycogen concentrations following the depletion protocol were associated with differences in pre-exercise phosphorylation status of key muscle cell signaling proteins that were generally ameliorated after the resistance exercise bout. Acute changes in translation initiation and glucose metabolism are stimulated by nutrient and contractile overload and mediated, at least in part, through the activation of the Akt-mTOR-S6K kinases (16, 38). We observed elevated resting Akt^{Ser473} phosphorylation in the LOW glycogen leg (Fig. 4*A*) but this disparity did not extend to the postexercise recovery period with similar responses between legs. In contrast, Creer and colleagues (11) reported similar Akt phosphorylation at rest and an attenuated postexercise response with low muscle glycogen. The discrepancies between studies may reflect differences in protocols employed for generating divergent glycogen concentration and the training status of the subjects, but is most likely related to the timing of postexercise biopsies. Nonetheless, it seems plausible that Akt-mediated signaling would be enhanced to promote glucose transport and glycogen resynthesis at rest due to low muscle glycogen, but strong contractile stimuli upregulates the metabolic response uniformly regardless of glycogen status.

As might be expected, differences in markers of glucose uptake glycogen synthesis and were observed at rest and postexercise (Fig. 5). Glycogen $synthase^{Ser641}$ dephosphorylation (activation) was significantly greater in LOW compared with NORM at every time point in the nutrient and placebo groups (Fig. 5*A*). Moreover, GS was significantly dephosphorylated in the LOW glycogen legs of both groups 1 h after the resistance exercise bout. Considering we previously showed no change in GS phosphorylation after resistance exercise (7), this may indicate that low glycogen concentration is a critical factor for the capacity of low-volume, high-intensity resistance exercise to exert any significant effect on glycogen synthase activity and (re)synthesis. Postexercise increases in AS160 phosphorylation were apparent with protein/CHO ingestion but were not different between NORM and LOW glycogen legs (Fig. 5*B*). Conversely, AS 160 phosphorylation increased postexercise only in LOW from the placebo group. This suggests any sensitivity AS 160 may exhibit to low glycogen availability following resistance exercise is eliminated upon adequate nutrient ingestion.

There was no effect of glycogen status on mTOR^{Ser2448} phosphorylation after resistance exercise in the placebo group, whereas nutrient ingestion elevated mTOR above rest 1 h after exercise to a greater extent in the NORM compared with the LOW leg (Fig. 4*B*). The increased phosphorylation of mTOR with protein/CHO ingestion likely represents a synergistic effect mediated through the insulin signaling cascade and capacity for amino acids to directly activate mTOR through a putative interaction between the Rag- and Rheb-GTPases (40). Although the disparity in the magnitude of mTOR phosphorylation may indicate a modest suppression due to low glycogen, there was still an ~4-fold increase in mTOR phosphorylation in the LOW leg that was sufficient to initiate activation of downstream proteins more proximal to translation initiation. Moreover, the sustained elevation in mTOR phosphorylation 4 h after resistance exercise in the nutrient group was similar between NORM and LOW legs.

The AMPK complex has a glycogen binding domain that may influence AMPK's role as a cell energy sensor while also having the capacity to negatively regulate mTOR activation (5, 20). Previous work demonstrated increased mTOR phosphorylation and muscle protein synthesis rates concomitant with elevated AMPK activity following exercise, indicating that any putative effect of AMPK on muscle protein synthesis in humans after resistance exercise may only be modest (14). Regardless, we failed to observe any changes in AMPK phosphorylation between legs in the placebo or nutrient groups that might explain the moderate difference in mTOR phosphorylation 1 h postexercise. Moreover, we demonstrate similar phosphorylation status of regulatory targets of mTOR proximal to translation initiation indicative of comparable activation despite disparity in glycogen concentration. This is in agreement with numerous previous studies investigating translational signaling that show increases in p70 S6K and rpS6 phosphorylation during the early recovery period following exercise and the augmented response with nutrient provision after an overnight fast (12, 13, 30).

Consistent with the changes in cell signaling, muscle mRNA responses of select genes associated with muscle proteolysis and catabolism were relatively unchanged by muscle glycogen concentration. Muscle atrophy F-Box (MAFbx; also known as atrogin-1) belongs to the ubiquitin proteasome pathway involved in tagging contractile protein for degradation by cellular proteosomes (4, 18), whereas myostatin is a putative negative regulator of muscle growth (41). The decrease in atrogin-1 mRNA abundance at 4 h postexercise in NORM and LOW legs in the placebo and nutrient groups (Fig. 7*A*) is similar to previous work from our laboratory showing a decrease in atrogin mRNA 3 h after resistance exercise (8). Likewise, the postexercise decrease in myostatin mRNA expression (Fig. 7*B*) is in accordance with previous studies that have examined mRNA changes following resistance exercise (22, 32). Interestingly, there was higher atrogin-1 and myostatin expression in the LOW glycogen leg compared with the normal glycogen leg after 4 h recovery in the nutrient group. To our knowledge, this is the first study to investigate the interaction of glycogen concentration and nutrients on catabolic genes after resistance exercise. The possibility exists that the abundance of exogenous CHO/amino acids and low muscle glycogen generated a signal to "switch on" processes regulating muscle remodeling/adaptation after exercise rather than preserving muscle protein by suppressing breakdown without postexercise nutrient provision in the placebo/fasted condition. However, it should be noted that the mRNA abundance of atrogin and myostatin were not elevated above resting levels and probably represents only a modest effect on catabolic processes.

In conclusion, and in contrast to our original hypothesis, commencing a bout of strenuous resistance exercise with low muscle glycogen availability failed to attenuate anabolic signaling and rates of myofibrillar protein synthesis compared with when the same exercise bout was undertaken with normal glycogen availability. Protein-CHO supplementation also failed to mediate any divergence in muscle protein synthesis between NORM and LOW during recovery. Moreover, whereas we observed some disparity between legs, undertaking exercise with low glycogen did not induce an increase in select mRNA markers of catabolic activity. Although we cannot rule out the possibility that alternative resistance training bouts employing different contraction volumes and intensities might generate a more pronounced effect of glycogen concentration on postexercise muscle cell signaling and muscle protein synthesis rates, our findings indicate that commencing resistance exercise with low muscle glycogen does not impair this anabolic response in the early recovery period. This is imperative when considering the potential for suboptimal muscle glycogen situations when undertaking multiple high-intensity exercise bouts in a day. Nonetheless, whereas low glycogen availability may promote the aerobic training phenotype, we provide new information to show that modulating glycogen concentration neither promotes nor inhibits the acute adaptation response after resistance exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.M.C., D.W.W., N.A.B., A.P.G., and V.G.C. performed experiments; D.M.C., D.W.W., N.A.B., S.M.P., and V.G.C. analyzed data; D.M.C. and V.G.C. interpreted results of experiments; D.M.C. prepared figures; D.M.C. and V.G.C. drafted manuscript; D.M.C., D.W.W., N.A.B., S.M.P., J.A.H., and V.G.C. edited and revised manuscript; D.M.C., D.W.W., N.A.B., S.M.P., J.A.H., and V.G.C. approved final version of manuscript; S.M.P., J.A.H., and V.G.C. conception and design of research.

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