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Influence of preexercise muscle glycogen content on transcriptional activity of metabolic and myogenic genes in well-trained humans

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Churchley EG, Coffey VG, Pedersen DJ, Shield A, Carey KA, Cameron-Smith D, Hawley JA. Influence of preexercise muscle glycogen content on transcriptional activity of metabolic and myogenic genes in well-trained humans. J Appl Physiol 102: 1604–1611, 2007. First published January 11, 2007; doi:10.1152/japplphysiol.01260.2006.—To determine whether preexercise muscle glycogen content influences the transcription of several early-response genes involved in the regulation of muscle growth, seven male strength-trained subjects performed one-legged cycling exercise to exhaustion to lower muscle glycogen levels (Low) in one leg compared with the leg with normal muscle glycogen (Norm) and then the following day completed a unilateral bout of resistance training (RT). Muscle biopsies from both legs were taken at rest, immediately after RT, and after 3 h of recovery. Resting glycogen content was higher in the control leg (Norm leg) than in the Low leg (435 ± 87 vs. 193 ± 29 mmol/kg dry wt; P < 0.01). RT decreased glycogen content in both legs (P < 0.05), but postexercise values remained significantly higher in the Norm than the Low leg (312 ± 129 vs. 102 ± 34 mmol/kg dry wt; P < 0.01). GLUT4 (3-fold; P < 0.01) and glycogenin mRNA abundance (2.5-fold; not significant) were elevated at rest in the Norm leg, but such differences were abolished after exercise. Preexercise mRNA abundance of atrogenes was also higher in the Norm compared with the Low leg [atrogin: ~14-fold, P < 0.01; RING (really interesting novel gene) finger: ~3-fold, P < 0.05] but decreased for atrogin in Norm following RT (P < 0.05). There were no differences in the mRNA abundance of myogenic regulatory factors and IGF-I in the Norm compared with the Low leg. Our results demonstrate that 1) low muscle glycogen content has variable effects on the basal transcription of select metabolic and myogenic genes at rest, and 2) any differences in basal transcription are completely abolished after a single bout of heavy resistance training. We conclude that commencing resistance exercise with low muscle glycogen does not enhance the activity of genes implicated in promoting hypertrophy.

adaptation; insulin-like growth factor I; atrogin; RING (really interesting novel gene) finger-1

IT IS GENERALLY ACCEPTED THAT for individuals involved in endurance sports, a chronic diet high in carbohydrate (CHO) enables athletes to train faster/harder or for a longer duration and thus achieve a superior training response (24). However, it has been proposed that a “cycling” of muscle glycogen stores may be desirable to further promote the training response/adaptation (8). In this regard, Hansen et al. (20) recently reported that untrained subjects who completed 10 wk of training, a portion (~50%) of which was commenced with low muscle glycogen levels, had a more pronounced increase in resting glycogen content and citrate synthase activity compared with when the same volume of training was undertaken with normal glycogen concentrations. Remarkably, this “train-low, compete-high” approach also resulted in a twofold increase in exercise time to fatigue compared with when subjects commenced all training sessions with normal glycogen levels. These authors suggested that under certain conditions, a lack of substrate (i.e., CHO) might trigger selected training adaptations that would be viewed as beneficial for performance (20). Certainly there is accumulating evidence to demonstrate that commencing endurance exercise with low muscle glycogen content enhances the transcription rate of a number of genes involved in the training adaptation (16, 28, 41). This is probably because several transcription factors include glycogen-binding domains, and when muscle glycogen is low, these factors are released and become free to associate with different targeting proteins (42).

In contrast to the upregulation of early-response genes when endurance exercise is commenced with low muscle glycogen stores, resistance exercise undertaken in a glycogen-depleted state may disrupt mechanisms involved in protein translation and blunt the normal adaptive response. Creer et al. (13) showed that when endurance-trained subjects commenced a bout of moderate-intensity resistance exercise with low (~175 mmol/kg dry wt) muscle glycogen content, phosphorylation of Akt, a critical signaling mediator of cell growth and metabolism (17), was diminished compared with when subjects undertook the same workout with normal (~600 mmol/kg dry wt) glycogen levels. Such responses would clearly be detrimental for athletes involved in resistance/strength exercise in which a major goal of training is muscle hypertrophy.

It is clear that altering substrate availability affects not only resting energy metabolism and subsequent fuel utilization during exercise but also many of the regulatory processes underlying gene expression (2, 21, 47). Therefore, the present study was undertaken to determine the effect of a bout of intense resistance exercise undertaken by trained athletes with either normal or low muscle glycogen content on the transcription of several early-response genes involved in the regulation of muscle mass. We hypothesized that preexercise muscle glycogen availability would result in differences in the basal transcriptional activity and exercise response of several genes that encode for proteins involved in the regulation of muscle growth.

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METHODS

Subjects

Seven male subjects [age 30 ± 6.7 yr, mass 94.4 ± 14.2 kg, maximal O2 uptake (VO2 max) 39.2 ± 3.7 ml·kg−1·min−1; means ± SD] who had been participating in regular strength/resistance training for 8.3 ± 5.8 yr and who did not participate in any form of endurance exercise volunteered for this investigation. Subjects were highly trained, as indicated by their maximal leg press dynamic strength [one repetition maximum (1 RM)] for each leg (~166 ± 18 kg). The experimental procedures and possible risks associated with the study were explained to each subject, who gave written informed consent before participation. The study was approved by the Human Research Ethics Committee of RMIT University.

Overview of Study Design

Diet and exercise manipulation ensured that subjects commenced a standardized bout of resistance exercise with normal muscle glycogen content in one leg (Norm) and low glycogen levels in the contralateral limb (Low). Muscle biopsies were taken from each leg at rest to assess the effects of starting muscle glycogen availability on mRNA abundance of genes associated with the regulation of metabolism and muscle mass, and they were taken during recovery from an intense resistance training bout to examine the effect of exercise on the transcriptional regulation of selected genes.

Preliminary Testing

VO2 max. Two-legged VO2 max was determined during an incremental test to exhaustion on a Lode cycle ergometer (Groningen, The Netherlands). The protocol has been described in detail previously (23). 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, subjects breathed through a mouthpiece attached to a Quark b2 metabolic cart (Hans-Rudolph syringe and gases of known concentration via enzymatic analyses with fluorometric detection (Jasco, East, MD)). A resting biopsy was taken using a 5-mm Bergstrom needle with suction applied (14). Approximately 100 mg of muscle were removed and immediately frozen in liquid N2.

Maximal strength. Maximal dynamic strength (1 RM) for each leg was determined on a CalGym leg press machine (Kalundura, Australia). Subjects completed the test with feet placed at the lowest edge of the plate and with the backrest at the lowest possible angle. Knee flexion angle was measured with a Jamar goniometer (Therapeutic Equipment, Clifton, NJ), such that ~110° of flexion was achieved during each repetition.

Familiarization to exercise training sessions. To familiarize subjects to one-legged cycling exercise (described subsequently), each subject completed three familiarization sessions in the 2–3 wk before the experimental trial. This session consisted of 2 × 10-min bouts of one-legged cycling, with a 2-min recovery period between repetitions. With each familiarization session, the power output that was sustained by subjects was gradually increased so that by the final sessions subjects were performing one-legged cycling at ~75% VO2 max (38).

Diet/exercise control. Subjects abstained from strenuous physical activity 72 h before an exercise depletion session (described subsequently) and also refrained from caffeine and alcohol consumption during this period. A high-CHO diet (~9 g CHO/kg) was consumed 36 h before the one-legged exercise depletion session. A qualified dietician assembled the diets, which were individualized to each subject’s food preferences. All food and drinks were supplied to subjects prepackaged. In addition, subjects were supplied with a food checklist to record their daily intake.

One-legged exercise depletion session. Subjects began the one-legged depletion session at an intensity equivalent to ~75% of two-legged VO2 max. The duration of each work bout was 10 min, with 2-min rest between work bouts. Subj ects maintained this work-to-rest ratio until volitional fatigue. At this time, power output was decreased by 10 W, and subjects rode 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 ER7100 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 liver glycogen stores and minimize any potential glycogen resynthesis in the Low leg, subjects then completed 30 min of arm cranking on a Monark Rehab Trainer 881E (Vansbro, Sweden). During the depletion session, subjects were allowed access to water ad libitum. Following this exercise depletion session, subjects were fed a low-CHO (~1 g/kg) evening meal.

Experimental testing session. Subjects reported to the laboratory at 0700–0800 after a 10- to 12-h overnight fast. After the subjects rested quietly in a supine position for 10 min, local anesthesia (2–3 ml of 1% Xylocaine, Astra Pharmaceuticals, Sydney, Australia) was administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis in preparation for muscle sampling. A resting biopsy was taken using a 5-mm Bergstrom needle with suction applied (14). Approximately 100 mg of muscle were removed and immediately frozen in liquid N2. At this time, two separate sites on each leg (~5 cm distal) were prepared for subsequent sampling.

Subjects then completed a standardized unilateral warm-up on the 45° leg-press machine and then rested for 10 min before the resistance exercise testing protocol was commenced. Resistance exercise consisted of eight sets of five repetitions at ~80% 1 RM for each leg. The Low leg began the protocol, with ~60 s rest before the Norm leg completed the same set. The next set for each leg began 3 min after the previous set was completed. Once the Low leg could not complete five full repetitions, the Norm leg would then replicate the same number of repetitions, and the weight was subsequently lowered by 5% for the following set. Immediately on completion of the last set of an exercise bout, a second biopsy was taken from the Low leg. After ~60 s rest, the Norm leg completed the last set, and the second biopsy was taken from this limb. Subjects then rested in a supine position for 3 h. At the end of the 3-h recovery period, during which subjects were only allowed access to water, a third muscle biopsy was taken from both legs. Every attempt was made to extract tissue from the same depth in the muscle, and muscle samples were frozen in liquid N2 within 10–15 s of extraction. Samples were stored at −80°C until subsequent analysis.

Analytical Procedures

Muscle glycogen concentration. A small piece of frozen muscle (40–50 mg) was removed and dissected free of all visible blood, connective tissue, and fat while immersed in liquid N2. This sample was then freeze-dried and powdered. One aliquot (~3 mg) of freeze-dried muscle was extracted and with 250 µl of 2 M hydrochloric acid incubated at 100°C for 2 h, and then neutralized with 750 µl of 0.667 M sodium hydroxide for subsequent determination of glycogen concentration via enzymatic analyses with fluorometric detection (Jasco FP-750 spectrofluorometer, Easton, MD). Glycogen concentration was expressed as milligrams of glycogen per kilograms of dry weight.

Total RNA isolation and reverse transcription. Frozen muscle (25–30 mg) was extracted by a modified acid guanidinium thiocyanate-phenol-chloroform extraction method for total RNA (9) described elsewhere (15). Total RNA extract was quantified using the RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). High quality of RNA was confirmed by the presence of ribosomal peaks with no additional signals (DNA contamination or RNA degradation) below the ribosomal bands and no shifts to lower fragments.
Extracted RNA (1 µg) was heated at 65°C for 10 min immediately before first-strand cDNA being generated using avian myeloblastosis virus reverse transcriptase (kit A3500, Promega, Madison, Wisconsin) with oligo(dT)₁₅ primer, in the presence of 1 mM of each dNTP and 20 U of recombinant RNasin ribonuclease inhibitor. The reaction was incubated at 42°C for 60 min and then terminated at 99°C for 10 min and 4°C for 5 min. The cDNA was stored at −20°C for subsequent analysis. Reverse transcription was performed for all samples simultaneously. Previous work in our laboratory has demonstrated minimal variation in efficiency when performed under these conditions (37).

**Primer design and mRNA quantification.** PCR primers were designed using Primer Express software version 2.0 (Applied Biosystems, Foster City, CA) from gene sequences obtained from GenBank (Table 1). Primer specificity was confirmed using the basic local alignment search tool. Primers were purchased from GeneWorks (Adelaide, Australia). Efficiency of PCR primers was confirmed by examining the dynamic range of responses for a series of dilutions of cDNA. Using the slopes of the lines, the efficiency (E) of each target amplification was calculated using the equation \(E = 10^{(-1/\text{slope})} - 1\). All primers used in this study demonstrated efficient amplification.

Real-time PCR was performed using the GeneAmp 5700 Sequence Detection System (Applied Biosystems). For the PCR step, reaction volumes of 20 µl contained 2× SYBRgreen PCR Master Mix (Applied Biosystems), forward and reverse primers and cDNA template (diluted 1:40). All samples were run in duplicate. The real-time PCR reaction was run for 1 cycle (50°C for 2 min, 95°C for 10 min) followed by 40 cycles (95°C for 15 s, 60°C for 60 s), and fluorescence emissions was measured after each of the repetitive cycles. A melting-point dissociation curve was generated to confirm that only a single amplification was calculated using the equation \(E = 10^{(-1/\text{slope})} - 1\).

A detailed analysis of muscle glycogen concentration changes was performed using the real-time PCR quantification method. Differences in mRNA levels were calculated using the comparative Ct method with cyclin D1 as an endogenous control. Gene expression was normalized using the 2^(-ΔΔCt) method, where ΔCt values were calculated for each gene of interest.

**RESULTS**

One-Legged Exercise Depletion Session Performance

The average time spent completing the one-legged depletion session at an intensity of ~75% of two-legged V\(\text{O}_2\)max was 91 ± 10 min. In addition, subjects also completed an average of ~25 ± 5 one-legged maximal effort sprint repetitions.

**Muscle Glycogen Concentration**

The effects of exercise and diet manipulation on muscle glycogen concentrations are shown in Fig. 1. As intended, the combination of the strenuous exercise depletion regimen and dietary manipulation resulted in a significantly lower resting glycogen level in the Low leg compared with the Norm leg (312 ± 129 vs. 102 ± 34 mmol/kg dry wt; P < 0.01). Resistance exercise significantly decreased glycogen content in both legs (P < 0.05), such that postexercise values remained significantly higher in the Norm than in the Low leg (312 ± 129 vs. 102 ± 34 mmol/kg dry wt; P < 0.01). However, net glycogen utilization as a result of the resistance training bout was not different between legs.

**mRNA Abundance**

GLUT4 and glycogenin mRNA abundance. As a result of differences in preexercise muscle glycogen concentrations (Fig. 1), GLUT4 mRNA abundance was significantly higher at rest in the Norm than the Low leg (P < 0.05, 0.7). Resistance exercise induced a threefold decrease in GLUT4 mRNA abundance (P < 0.01, ES 0.7) in the Norm leg but had little effect in the Low leg (Fig. 2A). Glycogenin mRNA abundance was threefold higher at rest in the Norm leg compared with the Low leg (ES 0.4), but this difference did not reach statistical significance. Resistance exercise had little effect on glycogenin mRNA abundance for either treatment condition (Fig. 2B).

Myogenin, MyoD, and IGF-I mRNA abundance. Although consistently elevated in the Norm leg compared with the Low leg, resting mRNA abundance for myogenin (ES 0.7), myogenic differentiation factor D (MyoD; ES 0.5), and IGF-I (ES 0.5) failed to reach statistical significance (Fig. 3). Divergent preexercise muscle glycogen content did not significantly alter the MyoD (ES Low 0.1, ES Norm 0.3) and IGF-I (ES Low leg 0.5, ES Norm leg 0.3) transcriptional response to resistance exercise. However, myogenin mRNA abundance was in-

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Table 1. Primer sequences and concentrations used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession Number</th>
<th>Forward Primer (5' → 3')</th>
<th>Reverse Primer (5' → 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrogin</td>
<td>NM_148177</td>
<td>CATCCCTATATGCACCTGTTGCTCAAAGA</td>
<td>TCCATTACACCCACATTTTAATG</td>
</tr>
<tr>
<td>MyoD</td>
<td>NM_002478</td>
<td>CCGCCATGACGAAATGAAATGA</td>
<td>GCAACCCTTGCTTTGGAT</td>
</tr>
<tr>
<td>Myogenin</td>
<td>NM_002479</td>
<td>GTGGCCCGCGAAGATG</td>
<td>TGGATGCCGCTCAGATGGA</td>
</tr>
<tr>
<td>MuRF</td>
<td>NP_115977</td>
<td>GGGCGTGGGCTCTGATTCTCCTT</td>
<td>TCTCCAAATTCCTCCTAGGGATT</td>
</tr>
<tr>
<td>IGF-I</td>
<td>NM_000875</td>
<td>CAGTGGTAAAGCCGAGATTTCA</td>
<td>TTCCTGTATAGTTGTCTCTCATAGATATC</td>
</tr>
<tr>
<td>Glycogenin</td>
<td>AH007114</td>
<td>CTATGGCCACAGCGTGTGTGAT</td>
<td>CTCCTCAGAATGCAGATG</td>
</tr>
<tr>
<td>GLUT4</td>
<td>NM_001042</td>
<td>CCACCTTGGCTCCCTCTCTC</td>
<td>TCCAAATGGCCTTCTCTAGCA</td>
</tr>
<tr>
<td>Myostatin</td>
<td>NM_005259</td>
<td>CAGAGAGAGAGAAGGCGGGGA</td>
<td>CAAAGACCAAAATGCGCTGGA</td>
</tr>
</tbody>
</table>

MyoD, myogenic differentiation factor D; MuRF, RING (really interesting novel gene) finger.
creased after exercise in the Low leg \((P < 0.05, \text{ES} 0.7)\) but not the Norm leg \((P = 0.4)\).

Atrogin, RING (really interesting novel gene) finger, and myostatin mRNA abundance. At rest, atrogin mRNA abundance was 14-fold higher in the Norm compared with the Low leg \((P < 0.01, \text{ES} 0.8; \text{Fig. 4A})\). Similarly, RING (really interesting novel gene) finger (MuRF) gene abundance was also higher (3-fold; \(P < 0.05, \text{ES} 0.6\)) in the Norm than in the Low leg at rest (Fig. 4B). Despite elevated myostatin transcriptional activity in the Norm leg compared with the Low leg at rest, this difference failed to reach statistical significance \((\text{ES} 0.4; \text{Fig. 4C})\). Resistance exercise resulted in a significant decrease in atrogin \((P < 0.05, \text{ES} 0.8)\) but not MuRF \((P = 0.5)\) or myostatin \((P = 0.2)\) mRNA abundance after 3 h of recovery in the Norm but not in the Low leg.

**DISCUSSION**

The acute responses elicited by a single bout of exercise, and the subsequent training-induced adaptations, are highly specific to the mode, intensity, duration, and frequency of that stimulus \((6, 22)\). In this regard, it is now generally accepted that the chronic adaptations to training represent the cumulative effect of repeated bouts of exercise \((10, 24, 50)\). Accordingly, it is possible to consider the molecular and cellular events that occur in skeletal muscle in response to an acute bout of exercise to gain an understanding of how training or other interventions (i.e., nutrition) might modulate these responses and promote (or inhibit) subsequent training adaptations. When such a molecular perspective on training adaptation is taken, any training-induced adaptation is a consequence of accumulation of specific proteins \((20)\). Accordingly, the altered gene expression that allows for these changes in protein concentration is pivotal to the training adaptation. Recent studies have demonstrated that endurance exercise induces transcription of several genes \((20, 35)\). Furthermore, it has been demonstrated that muscle glycogen is a determining factor for the transcription of some genes. Indeed, previous work has shown that exercising when muscle glycogen concentration was low resulted in a greater transcriptional activation of interleukin-6 \((20)\), pyruvate dehydrogenase kinase 4 \((20)\), hexokinase \((20)\), uncoupling protein 3 \((41)\), and heat shock protein 72 \((20)\) compared with when muscle glycogen concentration was normal (or high) at the start of exercise. Using exercise-diet manipulation to produce markedly different resting muscle glycogen concentration in each leg of the same subject, we provide novel data to demonstrate that glycogen content is a potent modulator of genes implicated in the regulation of muscle mass.

Few studies have investigated the interaction of muscle glycogen content and resistance training adaptation responses in skeletal muscle. Accordingly, before an acute bout of high-intensity resistance exercise, we employed a one-legged depletion protocol that was successful in creating a distinction in preexercise muscle glycogen concentration (Fig. 1). A major finding of the present study was that low preexercise muscle glycogen concentration reduced the mRNA abundance of genes implicated in the regulation of hypertrophy. Moreover, resting glycogen concentrations in the nondepleted leg resulted in elevated mRNA levels of the genes under investigation compared with the glycogen-depleted limb. While gene expression and subsequent protein synthesis are regulated at multiple steps, these results indicate attenuated expression of these genes when muscle glycogen content is low.

As might be expected, the mRNA abundance of genes regulating CHO metabolism were higher in the Norm than in the Low leg (Fig. 2). Greater CHO availability would likely favor enhanced activation of genes involved in glucose transport and glycogen synthesis. In support of this contention, we observed elevated GLUT4 and glycogenin mRNA in the non-
depleted leg. In addition, basal transcriptional activity of genes that promote muscle hypertrophy, including myogenic regulatory factors and insulin-like growth factor, was elevated in the Norm compared with the Low leg (Fig. 3). Although this greater mRNA abundance failed to reach statistical significance, ES calculations indicate a moderate-to-large effect that may have important physiological relevance. Work by Vissing and colleagues (49) has shown a two- to fourfold increase in MyoD mRNA levels following dietary manipulation. This finding, together with the results of the present study, suggests that MyoD gene expression is not only induced by contraction (1, 3, 31, 48, 52) but is also responsive to substrate availability (i.e., glycogen content). Similarly, myogenin has been implicated in a pathway regulating the metabolic status of skeletal muscle (27, 45, 49). The possibility exists that lower resting myogenin levels in the Low leg reflect reduced metabolic activity as a result of depleted muscle glycogen content. Regardless, in addition to their established function in regulating satellite cell activation, the altered mRNA abundance associated with divergent glycogen content suggests these transcription factors are sensitive to changes in macronutrient status and may have additional unconfirmed roles in skeletal muscle.

Surprisingly, we observed a greater mRNA abundance of genes associated with muscle atrophy in the Norm compared with the Low leg at rest (Fig. 4). These findings are difficult to reconcile with our current understanding of the regulation of skeletal muscle proteolysis. Atrogin, MuRF, and myostatin are key regulators of ubiquitin-mediated proteolysis that are up-regulated during skeletal muscle atrophy (5, 36). The evidence supporting their proposed role in proteolysis is compelling because the increase in gene expression of these ubiquitin proteins has been systematically induced with fasting, cachexia, diabetes, and disuse atrophy models (5, 26, 34, 36, 44). Consequently, it is reasonable to assume that increased expression of these atrophy genes would be exacerbated with low muscle glycogen concentration. However, an explanation for

![Fig. 3. Changes in mRNA abundance for myogenin (A), myogenic differentiation factor D (MyoD; B), and IGF-I (C), immediately before (rest) and 3 h after a standardized bout of resistance exercise (8 sets of 5 repetitions of 80% of 1 repetition maximum). Filled bars, exercise commenced with Norm conditions; open bars, exercise commenced with Low conditions. Values are means ± SD. # Significant difference between Low at rest and after 3-h recovery, P < 0.05.](image)

![Fig. 4. Changes in mRNA abundance for atrogin (A), RING (really interesting novel gene) finger-1 (MuRF; B), and myostatin (C), immediately before (rest) and 3 h after a standardized bout of resistance exercise (8 sets of 5 repetitions of 80% of 1 repetition maximum). Filled bars, exercise commenced with Norm conditions; open bars, exercise commenced with Low conditions. Values are means ± SD. * Significant difference between Norm and Low at rest, P < 0.01. † Significant difference between Norm at rest and after 3-h recovery, P < 0.05.](image)
the divergent atrophy gene response at rest might be that the high-intensity exercise undertaken during the depletion protocol attenuated the upregulation of atrophy pathways in the Low leg. Consequently, low-CHO feeding (~1 g/kg) followed by an overnight fast may have induced a greater atrophy response in the Norm compared with Low leg. Alternately, the downregulation of atrogin and MuRF transcription in the depleted leg may indicate an acute “fuel-sensing” adaptation response to low substrate availability that suppresses muscle proteolysis. Indeed, short-term fasting (40 h) in healthy subjects has failed to elicit an increase in the transcription of genes implicated in the regulation of muscle-specific atrophy (33). Of note, our laboratory has previously shown altered signaling and mRNA responses to divergent exercise in subjects with a prolonged training history in either endurance or strength training (10, 11). Accordingly, it may be that the transcriptional activity of these atrophy genes in the Low leg represents the early stages of skeletal muscle remodeling in response to a novel exercise stimulus or characterizes exercise-induced perturbation following unfamiliar contractile activity (i.e., cycling) in well-trained strength athletes. Regardless, further work is required to establish the effect of muscle glycogen concentration on the transcriptional activity of atrophy pathways.

A second novel finding of this study was the inability of high-intensity resistance exercise to override the effects of commencing exercise with low muscle glycogen and promote/inhibit transcription of genes implicated in the regulation of muscle mass. Moreover, in addition to the lack of mRNA response to exercise in the Low leg, elevated preexercise mRNA abundance with normal glycogen concentration was abolished 3 h after an acute bout of resistance exercise. A limitation of this study is the use of a single postexercise muscle sampling time point. We chose to sample muscle 3 h after the completion of an exercise bout because previous studies have reported that for the genes of interest in the present investigation, transcriptional activation occurs primarily during the initial few hours of recovery (19, 32, 43, 53). Nonetheless, our results indicate that a significant decrease in muscle glycogen concentration and lack of nutrient supply in the postexercise recovery period may impair the acute transcriptional activity of genes central to the regulation of muscle mass following resistance exercise.

Previous work has shown that endurance exercise undertaken with low muscle glycogen enhances the acute transcriptional response of selected metabolic genes and increases the performance capacity of skeletal muscle (20, 41). In contrast to these findings, we observed little increase in the mRNA abundance of genes that promote hypertrophy when commencing resistance exercise with low glycogen compared with normal glycogen content. Notably, Pilegaard and colleagues (41) observed an increase in mRNA abundance of genes associated with fat metabolism after 3-h low-intensity leg-kicking exercise undertaken with low muscle glycogen. Similarly, results from Hansen and coworkers (20) show an increase in mitochondrial enzyme 3-hydroxyacyl-CoA dehydrogenase and subsequent endurance exercise time to exhaustion following chronic training (10 wk) where exercise sessions were performed with low muscle glycogen. Differences in the metabolic role of the genes of interest and the intensity and duration of contractile overload in the present study may provide an explanation for divergent results of previous investigations. Furthermore, Blomstrand and Saltin (4) have previously shown that commencing 1-h cycling exercise (~75% \( \text{VO}_2 \text{max} \)) with low vs. high muscle glycogen concentration has little effect on postexercise protein synthesis. While there is a clear distinction between myogenic gene expression and protein synthesis per se, the results of Blomstrand and Saltin indicate that initiating exercise with low muscle glycogen does not appear to promote anabolic processes in skeletal muscle. Interestingly, myogenin mRNA abundance significantly increased after resistance exercise in the Low leg, whereas only a small effect was evident in the Norm leg, suggesting a greater “adaptive signal” in the Low leg (Fig. 3). Such an observation may, in part, have been due to increased metabolic stress with low substrate availability during high-intensity contractions (25). Although available evidence suggests endurance training with low muscle glycogen concentration may enhance metabolic adaptation and endurance performance in previously untrained individuals (20), the results from the present study indicate that undertaking high-intensity resistance training in a low-glycogen state does not appear to promote an anabolic response in trained athletes.

Resistance exercise represents a mode of training capable of inducing increases in muscle fiber cross-sectional area and compensatory hypertrophy (7). The hypertrophy response occurs when protein synthesis exceeds protein degradation (30, 46). Thus hypertrophy may result from increased protein synthesis and/or decreased protein degradation. Our present understanding of skeletal muscle proteolysis implicates ubiquitin-mediated proteolysis in the regulation of protein degradation. Atrogin and MuRF are key ubiquitin ligases that are upregulated during skeletal muscle atrophy (5, 18). Importantly, resistance training has been shown to both increase (29, 53) and decrease (10, 26, 51) the transcriptional response of myostatin, atrogin, and MuRF proteins in human skeletal muscle. An important finding of the present investigation was that following an acute bout of resistance training undertaken with normal muscle glycogen, we observed a significant decrease in the mRNA abundance of atrogin and a small effect in reducing MuRF and myostatin mRNA 3 h postexercise (Fig. 4). An important distinction between the present study and that of Yang and colleagues (53), who reported increases in transcriptional responses of several negative regulators of muscle growth, was the training status of the subjects. In contrast to sedentary participants, we utilized highly trained subjects with an extensive resistance training history. Phillips et al. (40) have previously shown attenuated protein breakdown and enhanced net protein balance in trained compared with untrained subjects at rest and during recovery from intense resistance exercise. Therefore, it is reasonable to suggest that an acute bout of heavy resistance training would induce substantial muscle damage in previously untrained individuals and promote inflammation and initiate activation of atrophy pathways. Conversely, the phenotype of highly trained athletes would likely reduce the activity of pathways promoting protein degradation both at rest and postexercise and decrease exercise-induced muscle damage and subsequent local inflammatory responses (40). Therefore, the findings of the present study provide further support for the putative capacity of chronic resistance training to downregulate pathways associated with proteolysis and atrophy, thereby promoting net protein synthesis.

In conclusion, we provide new information on the interaction of glycogen concentration and the transcriptional activity of contractile overload in the present study may provide an explanation for divergent results of previous investigations.
of metabolic and myogenic genes in skeletal muscle from strength-trained athletes. Our laboratory has previously reported that highly trained strength/power athletes require extensive overload to induce a significant increase in kinase activation and mRNA abundance compared with nonhabitual resistance trainers (10, 11). Thus innovative training interventions are required to further promote hypertrophy and inhibit atrophy pathways in these athletes. Accordingly, we utilized an extreme exercise-diet manipulation to compare skeletal muscle transcriptional activity at rest and following high-intensity resistance training with low or normal glycogen content. Our results demonstrate that 1) low muscle glycogen content has variable effects on the basal transcription of select metabolic and myogenic genes at rest, and 2) any differences in basal transcription are completely abolished after a single bout of heavy resistance training. We conclude that commencing resistance exercise with low muscle glycogen does not enhance the activity of genes implicated in promoting hypertrophy.

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REFERENCES


