Effect of consecutive repeated sprint and resistance exercise bouts on acute adaptive responses in human skeletal muscle

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Abstract

We examined acute molecular responses in skeletal muscle to repeated sprint and resistance exercise bouts. Six men (age 24.7 ± 6.3 yr, body mass 81.6 ± 7.3 kg, peak oxygen uptake 47 ± 9.9 mL·kg⁻¹·min⁻¹, one repetition maximum leg extension 92.2 ± 12.5 kg; mean ± SD) were randomly assigned to trials consisting of either resistance exercise (8 × 5 leg extension, 80% 1RM) followed by repeated sprints (10 × 6 s, 0.75 N·m torque·kg⁻¹), or vice-versa. Muscle biopsies from vastus lateralis were obtained at rest, 15 min after each exercise bout and following 3 h recovery to determine early signaling and mRNA responses. There was divergent exercise order-dependent phosphorylation of p70 S6K (S6K). Specifically, initial resistance exercise increased S6K phosphorylation (~75% P<0.05) but there was no effect when resistance exercise was undertaken after sprints. Exercise decreased IGF-I mRNA following 3 h recovery (~50%, P=0.06) independent of order, while MuRF mRNA was elevated with a moderate exercise order effect (P<0.01). When resistance exercise was followed by repeated sprints PGC-1α mRNA was increased (REX1-SPR2, P=0.02) with a modest distinction between exercise orders. Repeated sprints may promote acute interference on resistance exercise responses by attenuating translation initiation signaling and exacerbating ubiquitin ligase expression. Indeed, repeated sprints appear to generate the overriding acute exercise-induced response when undertaking concurrent repeated sprint and resistance exercise. Accordingly, we suggest that sprint-activities are isolated from resistance training and that adequate recovery time is considered within periodized training plans that incorporate these divergent exercise modes.

Keywords: repeated sprint ability, resistance training, adaptation.
Introduction

Concurrent training can be defined as the incorporation of two or more divergent exercise modes in a periodized training program. Since the classic work of Hickson (30), studies of concurrent training have attempted to determine the interaction of endurance and resistance exercise on subsequent adaptation in skeletal muscle. Given the distinct phenotypes generated in response to either chronic endurance or resistance training, and the apparent incompatibility of the exercise-induced adaptation (fatigue resistance versus high-force production), previous work in this area has endeavoured to elucidate the potential “interference” effect of concurrent training. In this regard, results of research investigating adaptation and performance changes when undertaking concurrent training has been equivocal (39, 40). While the specific physiological effects of concurrent training in skeletal muscle are far from established, work from our laboratory has revealed divergent responses for several molecular pathways when athletes undertake acute concurrent training (15). Indeed, we have shown that the proximity of endurance and resistance exercise reduced the extent of the desired molecular response and did not promote optimal activation of pathways to promote training specificity for each exercise mode.

An integral part of training programs for individuals participating in many sporting activities is repeated sprint ability. However, we currently lack information on the molecular training responses in skeletal muscle that occur in response to this type of activity. Sprint training promotes alterations in ion regulation and buffering capacity with little or no increase in muscle size (4, 21, 49). Moreover, high-intensity sprint exercise results in a large ionic disturbance, including the accumulation of muscle lactate and hydrogen ions (H⁺). Of note is that sprint exercise has recently been shown to induce a molecular adaptive profile associated with mitochondrial biogenesis and increased capacity for glucose and fatty acid oxidation (23). To the best of our knowledge, the molecular interactions of concurrent resistance and repeated sprint training have not been investigated. Indeed, such an approach represents a novel expansion of the conventional concurrent training paradigm. Consequently, we currently have no understanding of the potential mechanisms
that might underlie exercise-induced adaptation and enhanced/impaired muscle function with concurrent resistance and repeated sprint training. Despite the potential similarity in work-to-recovery ratio between sprint and resistance exercise, the greater rate of force production and inadequate recovery during repeated-sprints generates large disturbance to ion homeostasis, the accumulation of lactate, and altered substrate metabolism during ensuing repetitions that characterize a distinct exercise overload. (7). Thus, in contrast to the resistance exercise-induced increases in translation initiation and protein synthesis (65) repeated sprints might be expected to inhibit anabolic processes. Accordingly, the aim of the current study was to quantify the acute cellular responses in skeletal muscle when resistance and sprint training sessions were performed successively and to examine the effect of alternate exercise order (i.e. an initial bout of heavy resistance exercise, then repeated sprint exercise or vice versa). Given the paucity of data regarding the molecular response following repeated sprints in skeletal muscle we examined signaling associated with the translational machinery (Akt-mTOR-p70S6K) and changes in mRNA abundance related to anabolic growth (insulin-like growth factor [IGF]), proteasome-dependent protein breakdown (muscle RING finger [MuRF], Atrogin) and mitochondrial biogenesis/carbohydrate metabolism (peroxisome proliferator activated receptor gamma co-activator 1α [PGC-1α], hexokinase [HK]) (14). We also specifically chose this suite of molecular markers in order that we may be able to compare the responses to repeated sprints with our previous work showing concurrent resistance and endurance exercise-induced changes during the acute post-exercise recovery period (15). We hypothesized that the different exercise modes undertaken in close proximity would amplify the potential incompatibility of the acute molecular profile for each form of contractile activity.

Methods
Subjects

Six male subjects (age 24.7 ± 6.3 yr, body mass [BM] 81.6 ± 7.3 kg, peak oxygen uptake [VO2peak ] 47 ± 9.9 mL·kg⁻¹·min⁻¹, leg extension one repetition maximum [1-RM] 92.2 ± 12.5 kg; values
mean ± SD) who had been participating in regular concurrent resistance and endurance training (>3 × wk) volunteered for this study. The experimental procedures and possible risks associated with the study were explained to each subject who gave written informed consent prior to participation. The study was approved by the Human Research Ethics Committee of RMIT University.

Study Design

The study employed a randomized cross-over design where each subject completed two acute concurrent repeated sprint and resistance exercise sessions separated by a 2-wk recovery period, during which time subjects were instructed to continue their habitual physical activity. One experimental trial consisted of consecutive resistance then repeated sprint exercise bouts while the other trial was performed using the reverse exercise order i.e. repeated sprint then resistance exercise.

Preliminary Testing

Peak Oxygen Uptake (VO₂peak)

VO₂peak was determined during an incremental test to exhaustion on a Lode cycle ergometer (Groningen, The Netherlands). The protocol has been described in detail previously (29). In brief, subjects commenced cycling at a workload equivalent to 2 W·kg⁻¹ for 150 sec. Thereafter, the workload was increased by 25 W every 150 sec until volitional fatigue, defined as the inability to maintain a cadence >70 rev·min⁻¹. Throughout the test, subjects breathed through a mouthpiece attached to a metabolic cart (Parvomedics, Sandy, Utah) that was calibrated before testing using a 3-L Hans-Rudolph syringe and gases of known concentration (4.00% CO₂ and 16.00% O₂).

Maximal Strength

Quadriceps strength was determined during a series of single repetitions on a plate loaded leg extension machine until the maximum load lifted was established (1-repetition maximum [1-RM]). Repetitions were separated by 3 min recovery and were used to establish the maximum load/weight
that could be moved through the full range of motion once, but not a second time. Exercise range of motion was 85°, with leg extension endpoint set at -5° from full extension.

30 s Wingate

Wingate testing was undertaken on a Lode Excalibur Sport cycle ergometer (Groningen, The Netherlands). Subjects remained seated throughout the protocol which commenced with 60 s cycling at 75 W immediately followed by 30 s maximal sprint cycling against a resistance equal to 0.7 N·m torque·kg⁻¹ BM.

Diet/Exercise Control

Before an experimental trial (described subsequently), subjects refrained from training and other vigorous physical activity for a minimum of 48 h. Subjects were provided with standardized pre-packed meals that consisted of 3 g CHO·kg⁻¹ BM, 0.5 g protein·kg⁻¹ BM, and 0.3 g fat·kg⁻¹ BM consumed as the final caloric intake the evening prior to reporting for an exercise testing session.

Experimental Trials

An overview of the study testing protocol is shown in Figure 1. 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, local anaesthesia (2-3 ml of 1% Xylocaine (lignocaine)) was administered to the skin, subcutaneous tissue and fascia of the vastus lateralis of the subject’s leg in preparation for the series of muscle biopsies. A resting (basal) biopsy was taken using a 5-mm Bergstrom needle modified with suction. Approximately 150 mg of muscle was removed, blotted to remove excess blood with ~15-20 mg cut and placed in RNALater (Ambion, Austin, TX) and the remainder immediately frozen in liquid nitrogen. Subjects then completed the two exercise sessions (described in detail subsequently). Fifteen minutes after completion of the first exercise bout, a second biopsy was taken. Subjects then performed the second exercise bout. After 15 min recovery from the second exercise session, a third muscle biopsy was taken and subjects rested in the supine position until a fourth muscle biopsy was taken following the 3 h recovery period. Each muscle biopsy was
taken from a separate site and all samples were stored at –80 °C until subsequent analysis. In addition, blood samples (~2 mL) were taken at rest pre-exercise, 0, 5, 10 and 15 min after the first exercise bout, and 0, 5, 10, 15 and 30 min after the second exercise bout.

Resistance Exercise (REX)
Following a standardized warm-up (2 x 5 repetitions at 50% and 60% 1RM, respectively), subjects performed 8 sets of 5 repetitions at 80% 1-RM. Each set was separated by a 3 min recovery period during which the subject remained seated on the leg extension machine. Contractions were performed at a set metronome cadence approximately equal to 30°/s and strong verbal encouragement was provided during each set.

Repeated Sprint Exercise (SPR)
Subjects undertook 10 × 6 s maximal sprint efforts at a constant load. Subjects performed 10 maximal effort sprint cycling repetitions of 6 s duration against 0.75 N·m torque·kg⁻¹ BM on a Lode Excalibur Sport cycle ergometer (Groningen, The Netherlands). The initial sprint repetition was preceded by 60 s cycling at 75 W and subjects were instructed to initiate each sprint at 80 rpm and remain seated throughout the sprint exercise. Each sprint repetition was separated by 49 s passive rest followed by a 5 s rolling start for each subsequent sprint (i.e. each sprint started at 1 min intervals).

Analytical Procedures

Blood/muscle lactate and pH/H⁺
Whole blood was immediately analyzed for lactate concentration using an automated glucose/lactate analyzer (YSI 2300, Yellow Springs, Ohio). ~25 mg of frozen muscle was freeze-dried and blood/connective tissue was removed from the powdered muscle tissue. For determining muscle pH, samples (~3 mg) were homogenised on ice for 2 min in a solution containing sodium fluoride (NaF 10 mM) at a dilution of 30 mg dry muscle·mL⁻¹ of homogenising solution. The
muscle homogenate was then placed in a circulating water bath at 37º C for 5 min prior to and during the measurement of pH. The pH measurements were made with a microelectrode (MI-415, Microelectrodes Inc, Bedford, NH, USA) connected to a pH meter (SA 520, Orion Research Inc, Cambridge, MA, USA). For determining muscle lactate, samples (~4 mg) were enzymatically assayed using the spectrophotometry methods of Harris and co-workers (28).

**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, 50 mM NaF, 5 mM Na pyrophosphate, 1 mM DTT, 10 µg/mL trypsin inhibitor, 2 µg/mL aprotinin, 1 mM benzamidine and 1 mM phenylmethylsulfonyl fluoride. After determination of protein concentration (Pierce, Rockford, IL) lysate was re-suspended in Laemmli sample buffer, separated by SDS-PAGE, and transferred to polyvinylidene fluoride membranes blocked with 5% non-fat milk, washed with TBST (10 mM Tris HCl, 100 mM NaCl, 0.02% Tween 20) and incubated with primary antibody (1:1000) overnight at 4 ºC. Membranes were incubated with secondary antibody (1:2000) and proteins were detected via chemiluminescence (Amersham Biosciences, Buckinghamshire, U.K.; Pierce Biotechnology, Rockford, IL) and quantified by densitometry. Sample (50 µg) time-points for each subject were run on the same gel and quantified relative to alpha tubulin protein abundance. Polyclonal anti-phospho-mammalian target of rapamycin (mTOR) ser2448 (#2971); monoclonal anti-phospho-Akt ser473 (#9271), -tuberin (TSC2) thr1462 (#3617), -S6 ribosomal protein ser235/6 (#4856); anti-Akt (#9272), -TSC2 (#3635), -p70 S6K (#9202), -S6 ribosomal protein (#2217), -adenosine monophosphate kinase alpha (AMPKα) (#2603) were from Cell Signalling Technology (Danvers, MA). Anti-phospho-AMPKα thr172 was raised against AMPK alpha peptide (KDGEFLRpTSCGAPNY) as described previously (13) and anti-phospho-p70 S6K thr389 was from Millipore (#04-392; Temecula, CA). Monoclonal anti-α-tubulin control protein antibody was from Sigma-Aldrich (#T6074; St Louis, MO).
**Total RNA Extraction and RNA Quality Check**

Total RNA (23.1 ± 1.28 mg of tissue) was extracted in TRI reagent (Molecular Research Center, Cincinnati, OH). The quality and integrity [RIN of 8.56 ± 0.18 (SE)] of extracted RNA (0.28 ug/mg of tissue) was evaluated using an RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer.

**RT and Real-Time PCR**

Oligo (dT) primed first-strand cDNA was synthesized (150 ng total RNA) using SuperScript II RT (Invitrogen, Carsbad, CA). Quantification of mRNA (in duplicate) was performed in a 72-well Rotor-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). The current study was limited in that, to the best of our knowledge, no previous work has determined the suitability or otherwise of selected HK genes following repeated sprint exercise. We selected GAPDH based on a validation study from our laboratory which has shown GAPDH to be most stable in all human skeletal muscle fibre types following acute exercise (36, 66). All primers used in this study were mRNA specific (on different exons and over an intron) and were designed for SYBR Green chemistry using Vector NTI Advance 9 software (Invitrogen) (Table 1; sequences published elsewhere: peroxisome proliferator activated receptor gamma co-activator 1α [PGC-1α] (27), Atrogin-1, muscle RING finger [MuRF-1], forkhead box O 3a [Foxo3a] (43) and myogenic differentiation factor [MyoD] (56)). A melting curve analysis was generated to validate that only one product was present. The details regarding RT and PCR reaction parameters have been reported previously (43, 56).

**Relative qPCR data analysis**

Relative gene expression analysis comparing expression of a gene-of-interest (GOI) in relation to a reference-gene, based on the distinct cycle (Ct) differences, the delta Ct model (42), was used in this study as described in detail previously (56, 57, 68).

**Statistical Analysis**

We calculated sample size using the methods of Hopkins (32) and we have previously reported statistical significance for related physiological variables in cross-over studies employing 6-8
subjects (15-17, 52). All data were analyzed by two way repeated measures analysis of variance (two factor: time × exercise order) with Student-Newman-Kuels post-hoc analysis. Statistical significance was established when $P < 0.05$ (SigmaStat for windows Version 3.11). Lacking any information on the smallest substantial changes in acute responses to divergent exercise we performed additional post-hoc analysis in which each individuals data were back transformed against the mean of their corresponding resting pre-exercise values. For further detail of the statistical methods employed for magnitude based inferences the reader is referred to Hopkins and colleagues (33). Briefly, log transformed delta values between data time-points were directly compared and converted to Cohen effect sizes [ES] to determine the magnitude of change for each exercise bout and alternate exercise order (31). We chose a default confidence interval of 90% to calculate effect sizes via a spreadsheet making the same assumptions about sampling distributions that statistical packages use to derive P values (33). We interpreted the magnitude of the effect size by using conventional threshold values of 0.2 as the smallest effect, 0.5 as a moderate effect, and 0.8 as a large effect size (18, 33). Data are expressed as arbitrary units ± standard deviation (SD).

Results
Repeated sprint performance
There was a significant main effect for mean power output within the different sprint repetition bouts ($P<0.01$). Moreover, mean power declined ~11% (sprint 1-10) when repeated sprints were undertaken after resistance exercise (REX1-SPR2, 965 ±140 W vs. 860 ±106 W) and ~19% when sprints preceded resistance exercise (SPR1-REX2, 990 ±194 W vs. 804 ±76 W). However, mean power generated during the entire set of $10 \times 6$ s sprints was similar regardless of exercise order, with a significant difference between exercise orders only within sprint 4 ($P<0.05$).

Blood/muscle lactate and pH/H⁺
Changes in post-exercise blood lactate concentration resulted in differences for time and exercise order, and significant time × exercise order interactions ($P<0.01$; Figure 2). Specifically, when
resistance exercise preceded repeated sprints, blood lactate levels were only elevated above rest immediately after resistance exercise (REX1 0 min, ~2.4 ±1.5 mmol·L⁻¹, P<0.05; Figure 2). In contrast, blood lactate concentration was significantly elevated above rest at every time-point throughout the 15 min recovery when resistance exercise was undertaken after repeated sprints (REX2 ~3-4.7 mmol·L⁻¹, P<0.05; Figure 2). In addition, post-exercise blood lactate concentration was consistently increased above rest following repeated sprints regardless of exercise order (P<0.05; Figure 2).

There were significant main effects among the levels of time for muscle lactate concentration (Table 2). Repeated sprint exercise significantly increased muscle lactate above resting levels regardless of exercise order (P<0.05) and lactate concentration was higher following SPR1 compared with REX2 (P<0.05). Moreover, muscle lactate was only significantly elevated above rest following resistance exercise when preceded by repeated sprints (rest vs. REX2, P<0.05; Table 2). There were also significant main effects among the levels of time for both pH (rest vs. REX, rest vs. SPR, P<0.05) and H⁺ (rest vs. SPR, P<0.05; Table 2) but there were no significant interactions. The decrease in pH and increase in H⁺ was greatest when sprints were undertaken prior to resistance exercise compared with sprints performed subsequent to resistance exercise (Table 2).

Signaling responses

Main effects for Akt ser473 phosphorylation verged on significance for the different levels of time (P=0.055). The initial bout of exercise had little effect on phosphorylation of Akt regardless of contraction mode (Figure 3A). However, increased Akt phosphorylation 15 min after resistance exercise was undertaken subsequent to repeated sprints (SPR1-REX2) resulted in a large disparity compared with initial resistance exercise (REX2 vs. REX1 ~180%, ES >1.0; Figure 3A). Phosphorylation of Akt was largely unaffected 15 min after each bout of repeated sprints and phosphorylation state returned to resting levels 3 h following cessation of contractile activity regardless of exercise order.
Changes in TSC2\textsuperscript{thr1462} phosphorylation in response to resistance and repeated sprint exercise were modest (Figure 3B). There were minor changes to TSC2 phosphorylation following an initial bout of contractile activity regardless of mode, while a moderate increase was observed with the successive exercise bout (REX2 vs. REX1 \textasciitilde28\%, ES 0.5; SPR2 vs. SPR1 \textasciitilde56\%, ES 0.8; Figure 3B). There was a significant main effect for mTOR\textsuperscript{ser2448} phosphorylation 15 min following resistance exercise (P<0.05) while elevated phosphorylation above rest after repeated sprints approached significance (P=0.056; Figure 3C), but there were no significant interactions. Of note, there were corresponding mTOR phosphorylation responses to contractile activity regardless of exercise mode (Figure 3C). Elevated TSC2 and mTOR phosphorylation abated and returned to resting levels following 3 h recovery from the consecutive exercise bouts.

Diverse contractile activity and alternating exercise order induced divergent p70 S6K\textsuperscript{thr389} phosphorylation responses (P<0.05; Figure 3D). Specifically, an initial bout of resistance exercise generated an increase in S6K phosphorylation that was different from all other time-points within the REX1-SPR2 exercise order (P<0.05; Figure 3D). Moreover, repeated sprints did not promote phosphorylation of S6K and there was no effect when resistance exercise was undertaken after sprints, resulting in a significant order effect within resistance exercise bouts (REX1 vs. REX2 \textasciitilde74\%, ES >1.0, P<0.05). There was also disparity in delta S6K phosphorylation from rest following the 3 h recovery period with elevated phosphorylation observed when resistance exercise preceded repeated sprints (REX1-SPR2 \textasciitilde56\%, ES >1.0; Figure 3D). Changes in ribosomal protein (rp) S6\textsuperscript{ser235/6} phosphorylation resulted in a significant main effect within the different levels of time (P<0.05; Figure 3E). The enhanced phosphorylation of rpS6 15 min after initial resistance exercise was significantly different from rest and other post-exercise time-points (REX1-SPR2, P<0.05; Figure 3E). Of note, prior repeated sprints did not prevent increased rpS6 phosphorylation with subsequent resistance exercise but the magnitude of elevation from rest was moderately lower (REX1 vs. REX2 \textasciitilde50\%, ES 0.66). After 3 h recovery rpS6 phosphorylation abated but remained higher above rest following the resistance exercise-repeated sprints exercise order (REX1-SPR2 \textasciitilde69\%, ES>1.0; Figure 3E).
Changes in AMPK thr182 phosphorylation resulted in a significant main effect within the different levels of time (P<0.05; Figure 3F). There were similar increases in AMPK phosphorylation 15 min after an initial bout of contractile activity regardless of exercise mode. Moreover, the increase in phosphorylation of AMPK was sustained following the subsequent exercise bouts with no disparity in the magnitude of change from rest (Figure 3F). Following 3 h recovery AMPK phosphorylation was moderately higher above rest when repeated sprints preceded resistance exercise (SPR1-REX2 ~22% ES 0.53; Figure 3F).

mRNA responses

*IGF-I –MGF-MyoD*

Due to limitations in tissue sample size the mRNA analysis was restricted to comparisons at rest and 3 h after cessation of contractile activity. Comparable decreases in IGF-I mRNA from rest following 3 h recovery from exercise generated a large magnitude of effect (ES ~1.0) that verged on significance (~50%, P=0.06; Figure 4A). However, there was little difference in the magnitude of effect between the alternate exercise orders (-16%, ES 0.2). Similarly, there was a moderate decrease in MGF mRNA abundance 3 h post-exercise that was not significantly different from rest (~50%, ES ~0.7, P=0.08), with negligible disparity in the exercise order effect (-28% ES 0.26; Figure 4B). The elevation in MyoD mRNA 3 h after the cessation of contractile activity was large (ES >1.0) but failed to reach significance and was similar regardless of exercise order (~300%, P=0.06; Figure 4C).

*Atrogin-MuRF-Foxo3a*

Variation in individual responses resulted in little change in Atrogin and Foxo3a (Foxo3a not shown) mRNA abundance following the consecutive exercise bouts, with little discrepancy in the effect of the different exercise orders (ES <0.2; Figure 5A). In contrast, the mRNA abundance of MuRF was significantly elevated above rest following 3 h recovery from resistance exercise undertaken prior to repeated sprints, and also after sprints were performed before resistance exercise (ES >1.0, P<0.01; Figure 5B). The magnitude of increase in MuRF mRNA abundance was
moderately exacerbated when repeated sprints were undertaken subsequent to resistance exercise (REX1-SPR2 vs. SPR1-REX2, ~25% ES 0.75; Figure 5B).

**PGC-1α - HKII - TFAM - Citrate Synthase**

There was a significant main effect for the levels of time for PGC-1α mRNA in response to the combined exercise bouts (P=0.04; Figure 6A). The increased abundance of PGC-1α mRNA when repeated sprints preceded resistance exercise failed to reach significance despite a large effect (SPR1-REX2, ES >1.0, P=0.09), while the resistance exercise followed by repeated sprints exercise order was significantly different from rest (REX1-SPR2, ES >1.0, P=0.02). There was a moderate distinction in the relative increase above resting levels with augmented PGC-1α mRNA when repeated sprints followed resistance exercise (REX1-SPR2 ~35% ES 0.46; Figure 6A). HKII mRNA abundance was enhanced in response to contractile activity and was significantly elevated above rest after both exercise orders (ES >1.0, P<0.05; Figure 6B). The magnitude of increase in HKII mRNA 3 h post-exercise was moderately higher when repeated sprints were undertaken before resistance exercise (SPR1-REX2 ~46% ES 0.4; Figure 6B). There was disparity in individual TFAM and Citrate Synthase mRNA responses (data not shown) culminating in minor changes in mean mRNA abundance from rest following the 3 h recovery period.

**Discussion**

Individual training sessions provide the overload stimulus that generates specific adaptive events that ultimately alter cellular characteristics for enhanced performance capacity in skeletal muscle. We have recently established adaptive responses for several cellular and molecular pathways when athletes undertake consecutive acute resistance and endurance (concurrent) training (15). In the present study, we have taken a novel approach to the concurrent training paradigm by combining resistance exercise with repeated sprint performance to examine the acute specificity of training adaptation and exercise-order effects in skeletal muscle. Specifically, we show attenuated S6K and rpS6 phosphorylation following repeated sprints in an exercise order dependent manner. Moreover, repeated sprints also appeared to diminish the anabolic response to resistance exercise highlighted...
by a decrease in IGF-I mRNA and concomitant increase in MuRF mRNA abundance following 3 h recovery. In addition, we observed robust increases in the mRNA abundance of PGC-1α and Hexokinase with only a modest exercise order distinction, highlighting the capacity of high-intensity sprint exercise to stimulate acute mitochondrial and metabolic adaptive responses.

There were minimal differences in the decline of mean power despite a greater decrement with initial repeated sprints, likely due to higher initial power outputs and accompanying metabolic perturbations when undertaking sprints first (i.e. without any metabolic activity or residual fatigue from prior contractile activity) generating a slightly greater fatigue index (8). Moreover, during the 15 min recovery period following each repeated sprint bout blood lactate concentration was similar (Figure 2). However, the effect of elevated blood lactate levels following initial repeated sprint exercise resulted in higher concentrations after subsequent resistance exercise (REX2). This finding demonstrates that circulating blood lactate remained elevated during resistance exercise undertaken after sprints (Figure 2). Muscle lactate was only significantly elevated above rest following resistance exercise when preceded by repeated sprints (Table 2). Differences in muscle H⁺ content were less clear but the largest changes were observed following an initial bout of repeated sprints (Table 2). It may be reasonable to conclude that resistance exercise performed after the repeated sprint repetitions was undertaken in the presence of greater intramuscular and whole body metabolic acidosis compared with the initial resistance exercise bout. These findings provide support for the notion that the different exercise modes generate divergent stimuli that promote dissimilar signaling activity and gene expression. Thus, the close proximity to resistance exercise and metabolic changes as a consequence of the repeated sprints is significant given its potential to create an incompatible environment for resistance exercise-induced acute responses within the muscle milieu.

Akt has been characterized as a critical junction for multiple cell processes including glucose transport and hypertrophy in skeletal muscle (10, 64). In the present study changes in Akt-TSC2-
mTOR and AMPK phosphorylation and any subsequent exercise-order effect were modest. Following 15 min recovery from the initial exercise bout there was little divergence in Akt ser473 phosphorylation between exercise modes. In contrast, when resistance exercise was undertaken after sprints we observed elevated phosphorylation of Akt 15 min post-exercise, resulting in a disparity in the magnitude of effect from rest (ES >1.0; Figure 3A). To the best of our knowledge, this is the first study to examine the insulin/IGF signaling pathway in skeletal muscle following short repeated sprint activity. Accordingly, we present new data indicating repeated sprints do not appear to promote Akt-TSC2 mediated kinase activity. In support of this observation, Gibala and co-workers (23) have reported a decrease in Akt phosphorylation immediately (0 min) after 4 × 30 s all-out cycling bouts each separated by 4 min recovery. However, it should be noted that the acute post-exercise cell signaling time-course following both resistance and sprint exercise is currently unknown. Whether the disparity in Akt phosphorylation after a subsequent bout of resistance exercise in the present study represents the cumulative effect of the combined exercise bouts or is related to the time-course of activation remains to be established.

mTOR is a key regulatory protein for cell processes including translation and protein synthesis, and mitochondrial function (6, 19, 60). In the present study, there was a moderate increase in mTOR ser2448 phosphorylation from rest following the initial exercise bouts that was sustained with subsequent exercise regardless of mode and order (Figure 3C). The putative evidence for an Akt-independent mechanism capable of regulating mTOR phosphorylation in response to mechanical stretch (34, 35) may provide some rationale for the modest increases (main effects) in exercise-induced phosphorylation of mTOR without concomitant changes in Akt phosphorylation. We also observed similar changes in AMPK Thr172 phosphorylation independent of exercise order (Figure 3F). Given the potential for high-intensity contraction during repeated sprints to induce rapid changes in energy turnover and alter ATP:AMP ratio in skeletal muscle, a significant increase in AMPK activation might be expected. Indeed, Chen and colleagues (12) and Gibala and co-workers (23) have shown elevated AMPK activity immediately after 30 s sprints. The results of the present
study indicate that the 15 min recovery time-point may not have coincided with peak AMPK activity following the divergent contractile activity. Although few previous studies have measured both AMPK and mTOR phosphorylation/activity in vivo human skeletal muscle, Dreyer et al (20) have previously shown a significant increase in both AMPK activity and mTOR phosphorylation following a bout of resistance training. Mascher and colleagues (45) have also examined Akt-mTOR signaling after 60 min cycling and observed increased mTOR phosphorylation 0 and 30 min post-exercise, a time when AMPK activity is also elevated. In addition, work in a transgenic animal model by McGee and colleagues (47) provides support for the role of AMPK in promoting, rather than inhibiting, skeletal muscle growth independent of its role in metabolism. Clearly, much work remains to elucidate the interactions and subsequent physiological function of these signaling proteins in human skeletal muscle.

A novel finding of the present study was the divergence in S6K \textsuperscript{Thr389} and rpS6 \textsuperscript{Ser235/6} phosphorylation following the diverse exercise modes. The S6K and rpS6 proteins are proposed to regulate translational efficiency of mRNAs with a 5-terminal oligopyrimidine tract (‘TOP’) (59), although a causative relationship remains contentious (59), and increased phosphorylation of S6K and rpS6 is associated with the adaptive response to resistance exercise (17, 20, 22, 24). The mechanisms regulating these proteins are complex and Akt and mitogen activated protein kinase mediated signaling pathways may converge at rpS6 (58). Nonetheless, we (15) and others (5, 44) have also shown increased S6K/rpS6 phosphorylation after a single bout of endurance exercise in human skeletal muscle. In contrast, Gibala and colleagues (23) reported no change in S6K phosphorylation following multiple 30 s sprints. We provide new data indicating that short repeated sprint activity (10 x 6 s) does not promote translation initiation signaling in the early recovery period suggesting that the overriding acute response necessitates enhanced ion transport and buffering capacity (21, 49). The suppression of S6K/rpS6 phosphorylation when comparing resistance exercise undertaken after repeated sprints with initial resistance exercise is intriguing. In previous work from our laboratory we failed to see a significant exercise order effect and observed
increased S6K and rpS6 phosphorylation with consecutive endurance and resistance exercise bouts (15). Of note, metabolic acidosis has been shown to decrease protein synthesis in skeletal muscle of rodents and humans (11, 38). Thus, the elevated muscle lactate and low pH immediately prior to resistance exercise when undertaken after the initial repeated sprint bout implicates local acidosis as an inhibitor of resistance exercise-induced S6K and rpS6 phosphorylation (Table 2). Collectively, these results provide support for the putative capacity of an initial bout of repeated sprints to attenuate subsequent translation initiation up to ~3 h post-exercise, due at least in part to changes in the ionic state of the muscle milieu.

We also examined mRNA responses of select genes associated with hypertrophy, atrophy and metabolism in skeletal muscle (14) to provide an adaptive profile generated by the consecutive bouts of diverse exercise. Changes in mRNA abundance for genes of interest implicated in regulation of hypertrophy and atrophy did not reveal any obvious exercise order effect but the dual exercise bouts appeared to inhibit the IGF-I mRNA response and promote mRNA abundance of MuRF 3 h after cessation of contractile activity (Figure 4 and 5). A potential limitation of the present study was that muscle samples for equivalent 3 h post-exercise recovery time-points for each individual exercise bout were not taken. Nonetheless, our findings represent an adaptation “snapshot” 3 h after the cessation of contractile activity that characterizes the cumulative effect of the combined divergent stimuli.

IGF-I is a major growth factor that induces anabolic effects in skeletal muscle (3, 51, 62) and increased IGF-I expression is closely associated with muscle hypertrophy (1, 2). While recent work by Spangenburg and colleagues (63) offers putative evidence for IGF-I independent muscle hypertrophy, elevated mRNA abundance of IGF-I variants (IGF-IeA and MGF) corresponds with increases in muscle cross-sectional area following chronic exercise training (53). Few acute exercise studies have examined IGF-I mRNA and a range of IGF-I responses have been observed between 2-4 h recovery following resistance exercise (26, 48, 55). We have previously shown that following concurrent endurance and resistance exercise IGF-IeA and MGF mRNA abundance was
only attenuated when cycling immediately preceded resistance exercise (15). In contrast, the results of the present study revealed a decrease in IGF-I mRNA 3 h after the consecutive repeated sprint and resistance exercise bouts that was similar regardless of exercise order (Figure 4A and 4B). Thus, we suggest that repeated sprints may have a greater negative effect on growth factor mRNA abundance compared with an acute endurance exercise bout, and this effect is mediated by proximity per se rather than exercise order when combined with resistance exercise. Gene expression and resultant synthesis of new functional protein is regulated at numerous molecular “check-points”. While strong correlations between variance in mRNA and protein levels have been observed the possibility exists that within the complexity of adaptation in skeletal muscle changes in mRNA and protein may not always equate (41). However, more work is needed to clarify the acute effect of exercise-induced contraction and concurrent training on IGF-I mRNA responses.

We also observed similar mRNA changes in the myogenic differentiation factor (MyoD) in an exercise order-independent manner (Figure 4C). Yang and colleagues (67) have shown corresponding increases in skeletal muscle MyoD mRNA abundance following both endurance and resistance exercise. Likewise, previous work from our laboratory shows the cumulative effect of endurance and resistance exercise generates similar increases in MyoD mRNA abundance (15). Therefore, enhanced MyoD mRNA expression appears to be induced by a variety of contractile activity suggesting a minimal role in the specificity of training adaptation.

There were contrasting effects of the acute repeated sprint and resistance exercise bouts on the mRNA abundance of mediators of skeletal muscle inflammation and proteolytic responses in skeletal muscle (37, 61). Specifically, the cumulative effect of exercise induced a pronounced increase in MuRF mRNA, while variation in the individual response resulted in no alteration in mean Atrogin mRNA abundance (Figure 5A and 5B). There was only a moderate disparity in the increase above rest in MuRF mRNA with the alternate exercise orders (SPR1-REX2 vs. REX1-SPR2, ES 0.75). Atrogin and MuRF1 are E3 ubiquitin ligases implicated in skeletal muscle proteolysis via covalent tagging of specific proteins with ubiquitin for their subsequent degradation.
by the 26S proteasome (9, 25). We have seen a similar discrepancy in an exercise order-dependent manner which showed an increased magnitude in the MuRF mRNA response when an endurance exercise bout was performed following resistance exercise (15). Work by Louis and colleagues (43) indicates that MuRF mRNA abundance may be elevated 1-4 h after 30 min (75% \( VO_2_{\text{max}} \) running and 3 sets of 10 repetition (70% 1-RM) resistance exercise. Thus, the immediacy of repeated sprints in the present study did not attenuate the increase in markers of exercise-induced inflammation and may actually accentuate the muscle damage response following heavy resistance exercise. However, there is a paucity of data on the effect of repeated sprints on mRNA expression and acute concurrent training interactions, and more work is needed to elucidate the effect on anabolic and catabolic adaptation responses in skeletal muscle.

PGC-1\( \alpha \) has been described as a master controller of mitochondrial biogenesis and increases in PGC-1\( \alpha \) mRNA have been clearly evident following an acute bout of aerobic endurance exercise (16, 46, 50, 54). We have previously shown that an acute bout of concurrent endurance and resistance exercise incorporating 30 min cycling (70% \( VO_2_{\text{peak}} \) is insufficient to induce a robust increase in PGC-1\( \alpha \) mRNA abundance regardless of exercise order. In contrast, the results of the present study reveal a significant increase in PGC-1\( \alpha \) mRNA despite the proximity of the divergent exercise bouts and short cumulative duration of the sprint activity (60 s). Gibala and co-workers (23) previously showed only a small “dose” of intense exercise was sufficient to elevate PGC-1\( \alpha \) mRNA abundance. Likewise, our data highlight the capacity for repeated bouts of supra-maximal exercise to enhance acute PGC-1\( \alpha \) mRNA expression despite prior resistance exercise that may promote increased PGC-1\( \alpha \) protein content. Indeed, this unexpected revelation was achieved utilizing exercise of half the duration (60 s) to that of Gibala and colleagues (23). In addition, there was a modest disparity in the exercise order effect on PGC-1\( \alpha \) mRNA indicating that resistance exercise undertaken after repeated sprints may induce mild attenuation of the mitochondrial adaptive response but such an effect remains to be clearly established. Hexokinase mRNA abundance was increased 3 h after completing the alternate exercise bouts with only a modest order
effect demonstrating a comparable metabolic adaptive response of pathways regulating energy
 provision. Taken together, these results strongly suggest repeated sprints are capable of stimulating
 an initial acute response that may ultimately enhance skeletal muscle oxidative and metabolic
capacities when undertaken concurrently with resistance exercise regardless of exercise order.

Perspectives and Significance
This is the first study to examine the potential interference or additive effect of acute concurrent
training incorporating repeated sprint activity and resistance exercise in skeletal muscle. We chose
to employ successive exercise bouts with short recovery based on anecdotal reports from coaches of
elite athletes regarding selected training regimes in a number of sports, and also for the potential for
time-efficient adaptation with acute concurrent training sessions for health and fitness. A novel
finding of the present study is that repeated sprints appear to promote greater acute interference on
markers of resistance exercise adaptation than previously observed with endurance exercise (15).
Specifically, we observed an attenuated phosphorylation of kinases proximal to translation
initiation, and growth factor mRNA abundance. These acute responses may represent a temporary
suppression of molecular processes that promote net synthesis or maintenance of muscle mass.
Moreover, we also show elevated mRNA for a primary marker of inflammation/proteolysis that
would exacerbate the apparent negative effect on muscle mass, and these effects were largely
evident regardless of the order in which the consecutive exercise bouts were undertaken. An
additional finding of our study was that repeated sprints may provide a concentrated stimulus for
mitochondrial biogenesis and highlights the potential for short intense exercise to promote aerobic
adaptation responses. Collectively, our results indicate repeated sprint activity generates the
prevailing exercise-induced adaptive response and subsequently alters the acute adaptive profile in
skeletal muscle when undertaking concurrent repeated sprint and resistance exercise. Accordingly,
we suggest that repeated sprint activities are isolated from resistance training and that adequate
recovery time is considered within periodised training plans that incorporate these divergent
exercise modes.
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References


Table 1. Summary of mRNA primers for genes of interest (GOI) used in PCR reactions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Gene Bank No.</th>
<th>Amplicon Size (bp)</th>
<th>Annealing Temp. (°C)</th>
<th>mRNA Region (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>5’ 3’ CAGGGGCTTTTATTTCACAAAGCCA 5’ 3’ TGCGCAATACATCTCCAGCCTCCTTA</td>
<td>NM_000618</td>
<td>127</td>
<td>59</td>
<td>419-545</td>
</tr>
<tr>
<td>MGF (IGF-Ie)</td>
<td>5’ 3’ CAGAAGTATCAGCCCCCATCTACCAACAA 5’ 3’ TGCACTCCCTCTACTGCGTCTTCAA</td>
<td>NM_00111128</td>
<td>91</td>
<td>59</td>
<td>616-706</td>
</tr>
<tr>
<td>TFAM</td>
<td>5’ 3’ GAAAAACCAAAAAAGACCTGCTTTCAGCTT 5’ 3’ TTTTCTGCGGTAATCACCCT</td>
<td>NM_003201</td>
<td>86</td>
<td>59</td>
<td>589-674</td>
</tr>
<tr>
<td>HKII</td>
<td>5’ 3’ CAGCAGAAGACGCTGGAGAGAGCAT 5’ 3’ GTCAAACTCCTCGCGGTTGAGAT</td>
<td>MN_000189</td>
<td>126</td>
<td>60</td>
<td>3745-3870</td>
</tr>
<tr>
<td>CS</td>
<td>5’ 3’ CATGGACTGGCAATCAGGAAGTGCTT 5’ 3’ CTGGAAACAACCCGTCCTGAGTTGAG</td>
<td>MN_004077</td>
<td>144</td>
<td>58</td>
<td>1091-1220</td>
</tr>
</tbody>
</table>

*sense*: forward primer, *anti-sense*: reverse primer. IGF-I, insulin-like growth factor 1; MGF, mechano-growth factor; TFAM, mitochondrial transcription factor A; HKII, hexokinase II; CS, citrate synthase.
Table 2. Muscle lactate (La) and H⁺ content, and pH (mean ± SD) measured at rest and 15 min post-exercise following each exercise bout in the alternate exercise orders (REX, resistance exercise; SPR, repeated sprints).

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>REX1</th>
<th>SPR2</th>
<th>Rest</th>
<th>SPR1</th>
<th>REX2</th>
</tr>
</thead>
<tbody>
<tr>
<td>La (mmol·kg dw⁻¹)</td>
<td>9.2 ±4</td>
<td>15.8 ±4</td>
<td>28.2 ±16*</td>
<td>8.3 ±4.6</td>
<td>42.5 ±19*†</td>
<td>25.5 ±21*</td>
</tr>
<tr>
<td>pH</td>
<td>7.06 ±0.09</td>
<td>6.98 ±0.13</td>
<td>6.95 ±0.20</td>
<td>7.06 ±0.09</td>
<td>6.92 ±0.15*</td>
<td>6.99 ±0.12</td>
</tr>
<tr>
<td>H⁺ (mmol·kg dw⁻¹)</td>
<td>89 ±18</td>
<td>109 ±36</td>
<td>123 ±63</td>
<td>88 ±16</td>
<td>125 ±40</td>
<td>105 ±27</td>
</tr>
</tbody>
</table>

Significantly different \((P<0.05)\) versus * rest, † versus REX2.
Figure 1. Schematic of the experimental trials incorporating consecutive bouts of diverse exercise. The study utilized a randomized cross-over design were subjects completed both exercise orders (1-RM, one repetition maximum). The duration of each exercise bout was ~25 min vs. ~10 min for resistance and repeated sprint exercise, respectively.

Figure 2. Blood lactate at rest pre-exercise, immediately and at 5 min intervals during 15 min post-exercise recovery following each individual exercise bout (Ex 1 and 2) incorporating resistance exercise (REX; 8 × 5 leg extensions at 80% one-repetition maximum) and repeated sprints (SPR; 10 × 6 s maximal effort) in alternate exercise orders. Results are group means (± SD). Significant difference (P<0.05) versus * rest, † SPR2 vs. REX1, # REX2 vs. REX1, § REX2 vs. SPR1.

Figure 3. Phosphorylated Akt ser473 (panel A), tuberin thr1462 (TSC2; panel B) and mammalian target of rapamycin ser2448 (mTOR; panel C), p70 S6K thr389 (panel D), S6 ribosomal protein ser235/6 (S6; panel E) and adenosine monophosphate activated kinase α thr172 (AMPK; panel F) relative to α-tubulin at rest pre-exercise, 15 min after each individual exercise bout (Ex 1 and 2) and 3 h after cessation of training. Subjects completed two experimental trials incorporating consecutive resistance exercise (REX; 8 × 5 leg extensions at 80% one-repetition maximum) and repeated sprint (SPR; 10 × 6 s maximal effort) bouts performed in alternate order. Results are group means (± SD) and data are log-transformed values as arbitrary units. Significant difference (P<0.05) versus * rest, † SPR2, (†) REX2, ‡ 3 h.

Figure 4. Insulin-like growth factor 1 (IGF-I; panel A), mechano-growth factor (MGF; panel B), and myogenic differentiation factor (MyoD; panel C) mRNA abundance at rest pre-exercise and 3 h after cessation of training. Subjects completed two experimental trials incorporating consecutive resistance exercise (REX; 8 × 5 leg extensions at 80% one-repetition maximum) and repeated sprint (SPR; 10 × 6 s maximal effort) bouts performed in alternate order. Results are individual responses and group means in arbitrary units. Significant difference (P<0.05) versus * rest.
Figure 5. Atrogin (panel A) and muscle-ring finger (MuRF; panel B) mRNA abundance at rest pre-exercise and 3 h after cessation of training. Subjects completed two experimental trials incorporating consecutive resistance exercise (REX; 8 × 5 leg extensions at 80% one-repetition maximum) and repeated sprint (SPR; 10 × 6 s maximal effort) bouts performed in alternate order. Results are individual responses and group means in arbitrary units. Significant difference (P<0.05) versus * rest.

Figure 6. Peroxisome proliferator activated receptor gamma co-activator 1α (PGC-1α; panel A) and hexokinase II (HKII; panel B) mRNA abundance at rest pre-exercise and 3 h after cessation of training. Subjects completed two experimental trials incorporating consecutive resistance exercise (REX; 8 × 5 leg extensions at 80% one-repetition maximum) and repeated sprint (SPR; 10 × 6 s maximal effort) bouts performed in alternate order. Results are individual responses and group means in arbitrary units. Significant difference (P<0.05) versus * rest.
8 x 5 leg extension repetitions (80% 1-RM)
Duration = ~25 min

Resting Sample
Biopsy (BX)
Blood (*)

REX1

* * * Bx

SPR1

10 x 6 s maximal sprints (54 s recovery)
Duration = ~10 min

SPR2

* * * Bx

10 x 6 s maximal sprints (54 s recovery)
Duration = ~25 min

REX2

8 x 5 leg extension repetitions (80% 1-RM)
Duration = ~25 min

15 min recovery

15 min recovery

165 min recovery

15 min recovery

15 min recovery

165 min recovery
A) Atrogin mRNA (Arbitrary Units)

- Rest vs. 3 h

B) MuRF mRNA (Arbitrary Units)

- Rest vs. 3 h
- REX1-SPR2
- SPR1-REX2