Epigenetic Responses to Acute Resistance Exercise in Trained vs. Sedentary Men

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Abstract

Bagley, JR, Burghardt, KJ, McManus, R, Howlett, B, Costa, PB, Coburn, JW, Arevalo, JA, Malek, MH, and Galpin, AJ. Epigenetic responses to acute resistance exercise in trained vs. sedentary men. J Strength Cond Res 34(6): 1574–1580, 2020—Acute resistance exercise (RE) alters DNA methylation, an epigenetic process that influences gene expression and regulates skeletal muscle adaptation. This aspect of cellular remodeling is poorly understood, especially in resistance-trained (RT) individuals. The study purpose was to examine DNA methylation in response to acute RE in RT and sedentary (SED) young men, specifically targeting genes responsible for metabolic, inflammatory, and hypertrophic muscle adaptations. Vastus lateralis biopsies were performed before (baseline), 30 minutes after, and 4 hours after an acute RE bout (3 × 10 repetitions at 70% 1 repetition maximum [1RM] leg press and leg extension) in 11 RT (mean ± SEM: age = 26.1 ± 1.0 years; body mass = 84.3 ± 0.2 kg; leg press 1RM = 412.6 ± 25.9 kg) and 8 SED (age = 22.9 ± 1.1 years; body mass = 75.6 ± 0.3 kg; leg press 1RM = 164.8 ± 22.5 kg) men. DNA methylation was analyzed through methylation sensitive high-resolution melting using real-time polymerase chain reaction. Separate 2 (group) × 3 (time) repeated-measures analyses of variance and analyses of covariance were performed to examine changes in DNA methylation for each target gene. Results showed that acute RE (a) hypomethylated LINE-1 (measure of global methylation) in RT but not SED, (b) hypermethylated metabolic genes (GPAM and SREBF2) in RT, while lowering SREBF2 methylation in SED, and (c) did not affect methylation of genes associated with inflammation (IL-6 and TNF-α) or hypertrophy (mTOR and AKT1). However, basal IL-6 and TNF-α were lower in SED compared with RT. These findings indicate the same RE stimulus can illicit different epigenetic responses in RT vs. SED men and provides a molecular mechanism underpinning the need for differential training stimuli based on subject training backgrounds.

Key Words: skeletal muscle, myosin heavy chain, DNA methylation, IL-6, TNFα, AKT1

Introduction

A primary goal of strength and conditioning is to induce skeletal muscle adaptations that enhance human performance. Better understanding the mechanisms behind these alterations will likely improve physical training guidelines. The general process of cellular modification is universal and was first described by Francis Crick in 1958 (13). Termed the “central dogma of molecular biology,” Crick outlined this one-way process whereby DNA is transcribed into mRNA (gene expression) and subsequently translated into protein (13). Scientists now know that special upstream activities (collectively known as “epigenetics”) alter gene function without changing the actual nucleotide sequence (37). One of these epigenetic processes, referred to as DNA methylation, appears particularly important for exercise-induced structural remodeling of muscle (e.g., assembling new contractile proteins) (34), but it remains poorly understood.

DNA methylation refers to the addition of a methyl group to the 5 position of a cytosine nucleotide or 5-methylcytosine (5-mc) and is one of several epigenetic processes that influence the expression of genes (27). Although other forms of derivative DNA methylation exist (e.g., 5-hydroxymethylation), 5-mc DNA methylation is the most abundant form in the human genome (15). DNA methylation can occur throughout the genome but mainly does so at cytosines positioned next to a guanine nucleotide (“CpG” dinucleotide methylation). Groups of CpGs occurring in high density, called “CpG islands,” are particularly important for gene regulation (28). Increased methylation, especially in promoter or transcription start site regions, is associated with gene silencing or repression (28). DNA methylation is a semiperishable DNA modification and reactive to both gene and environmental inputs, making it particularly important to human health and function (53). Previous studies have shown that (a) DNA methylation is sensitive to a single bout of resistance exercise (RE) (45,47) and (b) changes in DNA methylation after acute exercise correspond to downstream expression of several genes (3) related to metabolic (30), inflammatory (11), and hypertrophic (7) adaptations. Moreover, a review of the literature indicates individuals with differing chronic exercise backgrounds have divergent basal (20,41) and postacute RE protein
expressions (19), but it is unclear whether this is driven by epigenetic modifications (45) or other factors (e.g., transcriptional or translational controls).

The purpose of this study was to compare changes in DNA methylation in response to acute RE in resistance-trained (RT) and sedentary (SED) young men, specifically targeting genes responsible for metabolic, inflammatory, and hypertrophic muscle adaptations. We hypothesized acute RE would have differential effects on gene-specific methylation in RT compared with SED that would reflect the known physiological adaptations within each respective group.

Methods

Experimental Approach to the Problem

Each subject visited the laboratory on 2 occasions separated by 3–7 days. For visit 1, subjects were assessed for anthropometric measures, maximal 1 repetition maximum [1RM] leg press, and leg extension strength, similar to previous studies (1,2,21). A 30-minute rest was given between the leg press and leg extension 1RM tests. During visit 2, muscle biopsies were performed before (baseline), 30 minutes, and 4 hours after an acute bout of moderate-volume, moderate-intensity RE.

Subjects

We operationally defined the RT group as men (n = 11) who were well trained and had engaged in heavy, structured, and continuous lower-body RE at least 2 times per week the past 12 months. Men were considered SED (n = 8) if they did not perform regular lower-body RE (or any other structured exercise program) the previous 12 months. Maximal strength and vastus lateralis (VL) muscle fiber type percentage were also used to confirm the training status of all subjects (2). All subjects were free of any lower-body, musculoskeletal, or neuromuscular injuries the previous 6 months. All subjects claimed no current or previous use of any anabolic steroid.

All subjects refrained from physical activity beyond activities of daily living and kept a detailed nutrition log for 72 hours, refrained from consuming alcohol and nicotine for ≥24 hours and caffeine for ≥12 hours, and drank ≥500 ml of water the night before and morning of visit 2 (51). The night before visit 2, an evening meal (Ensure Plus; Ross, Columbus, OH, USA) provided 50% of the estimated daily caloric requirements and standardized the amount, composition (28% fat, 58% carbohydrate, and 14% protein), sodium content, and timing of the final prebiopsy meal. The meal was consumed at ≥12 hours before the baseline biopsy, with no additional food, drink (except water), or caffeine consumed until after the final biopsies (4 hours) the following day. The California State University Institutional Review Board approved all procedures, and all subjects were informed of the benefits and risks before signing the approved informed consent document.

Procedures

Resistance Exercise Protocol. On arrival to the laboratory for visit 2, technicians collected nutrition logs, verbally confirmed adherence to the above instructions, and tested hydration through urine specific gravity. The baseline biopsy was completed and then followed by a standardized dynamic warm-up (same for all subjects and performed before exercise on both visit 1 and visit 2) (1,2,21). The RE consisted of leg presses for 1 × 10 repetitions at 50% 1RM and 3 × 10 repetitions at 70% 1RM (1-minute rest between sets). After 3–5 minutes of rest, 3 × 10 repetitions at 70% 1RM were completed on a leg extension machine (1-minute rest between sets). This protocol was chosen because it has been shown to produce a hypertrophic stimulus in numerous investigations.

Muscle Biopsies. After the overnight fast (≥12 hours), subjects rested supine for 30 minutes before undergoing a biopsy of the right VL. This initial biopsy was considered the control condition, and all subsequent changes in the muscle were compared with this baseline. Details of the biopsy procedure are described elsewhere (1,2,4,40,51). Briefly, a mark was made mid-muscle belly, cleaned with iodine, and subcutaneously anesthetized with 1% lidocaine/xylocaine (without epinephrine). A small incision was made, and then tissue (~100 mg) was obtained using the Bergström technique with suction. The muscle sample was immediately cleansed of excess blood, fat, and connective tissue, cut into multiple ~15-mg bundles, and either stored in cold skinning solution ([in millimeter]: 125 K propionate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl2, 20.0 imidazole [pH 7.0], and 50% [vol/vol] glycerol) at 4° C or rapidly frozen in liquid nitrogen and stored at ~80° C. The incision site was cleaned and covered with a sterile gauze and cohesive bandage tape. The procedure was repeated 30 minutes and 4 hours after completion of RE (biopsy location moved ~5 cm proximal each time) to investigate the epigenetic remodeling that precedes post-exercise gene expression in human skeletal muscle. During this time, subjects were given water ad libitum and allowed to use computers, television, and their cell phones. Only light activity (e.g., walking to the restroom) was permitted, whereas sleeping and consuming food were not.

Myosin Heavy Chain Fiber Type Identification. Methods for single muscle fiber typing through sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis have been published previously (1,2,4,40,51). Briefly, ~100 individual muscle fibers per biopsy sample were randomly selected and extracted longitudinally in a physiological buffer using fine-tipped tweezers under a light microscope at room temperature and placed into 80-µl SDS buffer (10% SDS, 6 mg·ml⁻¹ EDTA, 0.06 m Tris [pH 6.8], 2 mg·ml⁻¹ bromophenol blue, 15% glycerol, and 5% β-mercaptoethanol). Aliquots were loaded into individual wells in a 3.5% loading gel and 5% separating gel and run at 5° C for 15.5 hours (SE 600 Series; Hoefer, San Francisco, CA, USA). Gels were subsequently fixed, rinsed, and silver stained to reveal the myosin heavy chain (MHC) isoform expressed in each individual fiber (according to migration distance). Fibers were identified as MHC I, MHC IIa, MHC IIb, or MHC IIx.

DNA Methylation Assessment. A panel of genes was selected to undergo analysis of DNA methylation through methylation sensitive high-resolution melting (MS-HRM) using real-time polymerase chain reaction. Long interspersed nuclear element 1 (LINE-1) was used as a surrogate for global methylation with previously designed and validated primer sets (52). Gene-specific primer sets were designed to analyze regional DNA methylation in the promoter region within 500 bp of a transcription start site for the following genes: Glycerol-3-Phosphate Acyltransferase 1 (GPAM), Sterol Regulatory Element Binding Transcription Factors 1 and 2 (SREBF1 and SREBF2), Interleukin 6 (IL-6), Tumor Necrosis Factor Alpha (TNFa), Mechanistic Target Of Rapamycin Kinase (MTOR), and AKT Serine/Threonine Kinase 1 (AKT1). Primers were designed with MethPrimer 2.0 according to recommendations for MS-HRM analysis (33,54,55).
Gene-specific primers along with details regarding the areas of the genome analyzed are given in Supplemental Digital Content 1 (see Table, http://links.lww.com/JSCR/A133). Polymerase chain reactions were performed on a Roche LightCycler 480ii with High-Resolution Melting Master according to the manufacturer’s specifications (Roche Life Science, Penzberg, Germany). Each reaction was performed in triplicate with positive controls (Qiagen methylated DNA [Hilden, Germany] mixed in ratios from 0 to 100%), negative controls, and genomic template controls. Gene Scanning Software on the Roche Lightcycler 480ii was used to analyze high-resolution melt profiles and produce difference curves relative to the 0% methylated control. Area under the curve was calculated from extracted difference curve data in Excel, and percent methylation for each reaction was estimated based on a standard curve from the positive controls. Methylation sensitive high-resolution melting provides regional methylation quantification comparable with the gold standard of pyrosequencing (49).

Statistical Analyses
Separate t-tests were used to analyze potential differences in anthropometric, strength, and nutrition variables. Fiber type was assessed through a 2 (group: RT or SED) × 6 (fiber type: MHC I, I/IIa, IIa, IIa/IIX, IIX, and I/IIX/IIa) analysis of variance (ANOVA). Separate 2 (group: RT or SED) × 3 (time: baseline, 30 minutes, and 4 hours) repeated-measures ANOVA were performed to examine potential changes in DNA methylation for each of the target genes. When appropriate, a post hoc Tukey test was performed to identify mean differences (35). In cases where the baseline value for a specific gene was statistically different between the 2 groups, an analysis of covariance (ANCOVA) was used with the baseline DNA methylation values as the covariate (35). Statistical significance was set at p ≤ 0.05, and the data were analyzed using the Statistical Package for the Social Sciences software (v. 22.0, IBM SPSS, Armonk, NY, USA).

Results
Anthropometrics, Muscle Strength, and Nutritional Intake
Resistance-trained men were significantly older and stronger in both the leg press and leg extension, and tended to have lower body fat than SED (Table 1). Resistance-trained men also consumed significantly more calories, fat, and protein, but not carbohydrates, than SED.

Myosin Heavy Chain Fiber Type
A total of 1,875 single muscle fibers were analyzed for MHC type (n = 97 ± 7 per RT; n = 101 ± 3 per SED). Resistance-trained men had significantly more MHC IIa (p < 0.001) and fewer IIa/IX (p < 0.001), IIX (p = 0.011), and I/IIX/IIa (p < 0.001) myofibers than SED (Figure 1).

DNA Methylation of Target Genes
Global Methylation. The mixed-factorial ANOVA for LINE-1 revealed a significant group × time interaction [F(2,30) = 4.07; p = 0.027] and a significant main effect for time [F(2,30) = 11.314; p < 0.001]. However, there was no significant main effect for group [F(1,15) = 0.418; p = 0.528]. The follow-up analysis to the significant interaction indicated no significant mean differences (p > 0.05) between the 2 groups for each corresponding time point (i.e., RT-30 minutes vs. SED-30 minutes). The change in percent methylation across the 3 time points was significant for RT, but not SED (Figure 2).

Metabolic Genes. The mixed-factorial ANOVA for GPAM revealed a significant group × time interaction [F(2,32) = 4.470; p = 0.019] and a significant main effect for group [F(1,16) = 15.732; p = 0.001]. However, there was no significant main effect for time [F(2,32) = 0.244; p = 0.785]. The follow-up analysis to the interaction indicated significant mean differences between groups for 2 of the 3 time points (Figure 2). In addition, there was a significant mean difference for percent methylation between RT-baseline and RT-4 hours (Figure 2).

The mixed-factorial ANOVA for SREBF2 revealed a significant group × time interaction [F(2,32) = 5.145; p = 0.012], but no significant main effects for time [F(2,32) = 0.826; p = 0.447] and group [F(1,16) = 1.182; p = 0.293]. The follow-up analyses indicated significant mean differences in percent methylation between the 2 groups for the 4 hours post-exercise time point. Moreover, within the SED group, percent methylation was significantly lower 4 hours post-exercise vs. baseline and 30 minutes post-exercise. In addition, the mixed-factorial ANOVA for SREBF1 revealed no significant group × time interaction [F(2,34) = 0.643; p = 0.532] or main effects for time [F(2,34) = 1.193; p = 0.316] and group [F(1,17) = 0.165; p = 0.690] (Figure 2).

Inflammatory Genes. For the 2 genes associated with inflammation (Figure 2, IL-6 and TNF-α), there were significant mean differences between the 2 groups at baseline (hypomethylated in SED). Therefore, to determine changes in methylation patterns after our intervention, we used the baseline value as a covariate (35). Before performing the ANCOVA, we tested the homogeneity of regression assumption that was met for both IL-6 (p = 0.482) and TNF-α (p = 0.071) (35). For IL-6, the mixed-factorial ANCOVA revealed no significant group × time interaction [F(1,15) = 0.123; p = 0.731] or main effects for time [F(1,15) = 0.277; p = 0.607] and group [F(1,15) = 1.61; p = 0.224]. For TNF-α, the mixed-factorial ANCOVA revealed no interaction [F(1,15) = 0.363; p = 0.556] or main effects for time [F(1,15) = 2.83; p = 0.113] and group [F(1,15) = 0.031; p = 0.862].

Hypertrophic Genes. We also examined 2 genes associated with pathways for skeletal muscle hypertrophy (mTOR and AKT1). For mTOR, the mixed-factorial ANOVA revealed no significant

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics and self-reported daily nutritional information.†</th>
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<tr>
<td></td>
<td>Resistance trained (RT)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>26.1 ± 1.0</td>
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<tr>
<td>Height (cm)</td>
<td>175.7 ± 2.0</td>
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<tr>
<td>Body mass (kg)</td>
<td>84.3 ± 0.2</td>
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<td>Body fat (%)</td>
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<td>Training history (y)</td>
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<td>Leg press 1RM (kg)</td>
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<td>Leg extension 1RM (kg)</td>
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<tr>
<td>Daily nutrition</td>
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<td>137.2 ± 24.6</td>
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<tr>
<td>Protein (g)</td>
<td>161.3 ± 13.0</td>
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†Mean ± SEM

1RM = 1 repetition maximum.
group × time interaction \([F(2,34) = 0.145; p = 0.866]\) or main effects for time \([F(2,34) = 0.502; p = 0.610]\) and group \([F(1,17) = 1.29; p = 0.272]\). For \textit{AKT1}, the mixed-factorial ANOVA revealed no significant group × time interaction \([F(2,30) = 2.80; p = 0.077]\) or main effect for time \([F(2,30) = 0.340; p = 0.715]\). However, there was a significant main effect for group \([F(1,15) = 12.99; p = 0.003]\), which indicated that overall SED had a significantly lower percentage of DNA methylation than RT (5.7 ± 1.0 vs. 10.6 ± 0.9%) (Figure 3).

\section*{Discussion}

The present investigation aimed to determine whether habitual exercise history differentially influenced epigenetic responses in muscle after high-intensity acute RE. To accomplish this, we focused on key genes involved in metabolism (\textit{GPAM}, \textit{SREBF1}, and \textit{SPREBF2}), inflammation (\textit{IL-6} and \textit{TNF-\alpha}), and hypertrophy (\textit{mTOR} and \textit{AKT1}). These 3 areas were purposefully selected because long-term resistance training has been shown to improve insulin sensitivity and other metabolic variables (23), enhance anti-inflammatory responses (9), and activate signaling pathways associated with hypertrophy (44). To the best of our knowledge, this is the first investigation to assess skeletal muscle epigenetic responses (DNA methylation) in RT and SED young men after acute RE. The main finding was genes associated with metabolism (but not inflammation or hypertrophy) were significantly influenced by training status. These data add to the knowledge base of the myriad mechanisms responsible for muscle remodeling with exercise.

The \textit{GPAM} (glycerol-3-phosphate acyltransferase) gene codes for a mitochondrial enzyme that is responsible for the rate-
Inflammation plays a critical role in skeletal muscle remodeling and is influenced by various factors, including exercise and the post-exercise recovery period (24). IL-6 and TNFα are 2 important modulators of the inflammatory response. Both are considered proinflammatory cytokines; however, IL-6 may have beneficial effects as an anti-inflammatory myokine (i.e., signaling molecule in the muscle) (39). The IL-6 and TNFα genes were hypomethylated at baseline in SED compared with RT, suggesting SED had a higher expression of these genes at rest. IL-6 protein levels increase with acute exercise, which is consistent with the hypomethylation after exercise found in SED in the current study (17). These results indicate a chronic adaptation occurred in RT that potentially created a gene methylation profile better suited to recover from repeated bouts of exercise by lowering the basal expression of proinflammatory mediators. Nevertheless, when controlled for the baseline values, there were no significant changes in DNA methylation between groups (Figure 2). Future work should aim to understand the effects of multiple RE bouts on inflammatory gene methylation over time, and the associated anti-inflammatory vs. proinflammatory effects of these gene changes. This may lead to the development of a biomarker that could be used to assess “molecular” muscle fatigue in an athlete.

Another metabolic gene, SREBF2 (sterol regulatory element binding transcription factor 2), codes for a transcription factor that controls sterol-regulated genes involved in cholesterol and lipid homeostasis (48). Sterol-regulated binding proteins are highly expressed in adipocytes and the liver (25,36). Moreover, this gene plays an important role in skeletal muscle function and is responsive to a wide range of stimuli (12,14,32). Similar to GPAM, post-RE SREBF2 methylation decreased in SED but increased in RT (Figure 2). This supports previous research, which showed excessive, damaging RE (300 eccentric repetitions) upregulated SREBF2 (38). The hypomethylation of GPAM and SREBF2 in SED indicates they experienced greater metabolic overload compared with RT in response to the same relative exercise demand.

The objective of this study was to provide an initial exploration of DNA methylation activities important to RE-induced skeletal muscle adaptions in RT and SED individuals. Few studies to date have broached this topic (3,42,47), and many variables not yet studied (such as biopsy timing and measurement methods) may have influenced our findings. For example, we found no immediately post-exercise (30 minutes) changes in global methylation with the widely used surrogate marker of LINE-1. By contrast, Barrés et al (3) used a luminometric methylation assay and found a decrease in global methylation 20 minutes after acute aerobic exercise in sedentary individuals. Thus, it is possible LINE-1 responds on a unique time-course, or these 2 assessment methods of providing a “snapshot” of global methylation may not entirely overlap. The 4-hour time point did reveal significant hypomethylation in RT, but not SED, indicating chronic RE may downregulate markers of skeletal muscle LINE-1 activity (45).

Our subjects also possessed significant differences in both nutritional practices and muscle fiber type compared with previously...
studies populations. The latter appears particularly important, as epigenetic pattern is known to differ significantly between specific fiber types (5). However, the paucity of research in this area in human muscle preclude us from determining how (and to what magnitude) training history, nutrition, and fiber type specifically influenced each epigenetic marker of metabolism, inflammation, and hypertrophy. Therefore, future studies should (a) use epigenomic strategies to survey the full methylome, (b) further explore the timeline of post-exercise responses, and (c) examine functional methylation changes after acute and chronic exercise in a fiber type-specific fashion while isolating the numerous other factors that likely influence epigenetics (e.g., nutrition, age, exercise history, sex, sleep, health status, exercise type, etc.). Such data would greatly enhance our understanding of how epigenetics influence gene and protein expression/activation and eventual cause changes in whole muscle structure, function, and performance.

**Practical Applications**

The current investigation highlights how the same acute RE stimuli can induce significantly different skeletal muscle epigenetic modifications in previously RT compared with sedentary individuals. Most guidelines advise practitioners to implement slightly lower RE volume and intensity when coaching untrained individuals (26). Our data appear to support such conclusions, providing a molecular mechanism underpinning the need for differential training stimuli based on subject training backgrounds. However, extensive work is needed before further actualizing this RE-induced epigenetic signature knowledge into practical recommendations and programming decisions.

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