Effect of Resistance Exercise Intensity on the Expression of PGC-1α Isoforms and the Anabolic and Catabolic Signaling Mediators, IGF-1 and Myostatin, in Human Skeletal Muscle

Journal:	*Applied Physiology, Nutrition, and Metabolism*
Manuscript ID	apnm-2016-0047.R1
Manuscript Type:	Article
Date Submitted by the Author:	19-Mar-2016
Complete List of Authors:	Schwarz, Neil; University of South Alabama, HPELS McKinley-Barnard, Sarah; Baylor University Spillane, Mike; University of South Alabama, HPELS Andre, Thomas; Baylor University Gann, Joshua; Baylor University Willoughby, Darryn; Baylor University,
Keyword:	resistance exercise < exercise, myostatin, PGC-1α, IGF-1, skeletal muscle

SCHOLARONE™
Manuscripts

Effect of Resistance Exercise Intensity on the Expression of PGC-1α Isoforms and the Anabolic and Catabolic Signaling Mediators, IGF-1 and Myostatin, in Human Skeletal Muscle

Neil A. Schwarz[1], Sarah K. McKinley-Barnard, Mike B. Spillane[1], Thomas L. Andre, Joshua J. Gann, & Darryn S. Willoughby

Corresponding Author:

Darryn S. Willoughby
Exercise and Biochemical Nutrition Laboratory
Department of Health, Human Performance, and Recreation
One Bear Place #97313
Baylor University
Waco, Texas 76798-7313
Phone: 254-710-3504
Fax: 254-710-3527
Email: darryn_willoughby@baylor.edu

Contact information:

Neil A. Schwarz (neilschwarz@southalabama.edu)
Sarah K. McKinley-Barnard (sarah_mckinley@baylor.edu)
Mike B. Spillane (micheilspillane@southalabama.edu)
Thomas L. Andre (thomas_andre@baylor.edu)
Joshua J. Gann (joshua_gann@baylor.edu)

Affiliation of all authors at time of study:

Exercise and Biochemical Nutrition Laboratory
Department of Health, Human Performance, and Recreation
One Bear Place #97313
Baylor University
Waco, Texas 76798-7313

[1]Current affiliation of Neil A. Schwarz and Mike B. Spillane:

Department of Health, Physical Education, and Leisure Studies
University of South Alabama
307 N. University Blvd.
Mobile, AL 36688

1

Abstract:

The purpose of this study was to investigate the acute mRNA expression of the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) isoforms, insulin-like growth factor-1Ea (IGF-1Ea), and myostatin in response to two resistance exercise intensities. In a uniform-balanced, cross-over design, ten participants performed two separate testing sessions involving a lower-body resistance exercise component consisting of a lower-intensity (50% of 1-repetition maximum; 1-RM) protocol and a higher-intensity (80% of 1-RM) protocol of equal volumes. Muscle samples were obtained at before exercise (PRE), 45 minutes, 3 hours, 24 hours, and 48 hours post-exercise. Resistance exercise did not alter total PGC-1α mRNA expression; however, distinct responses of each PGC-1α isoform were observed. The response of each isoform was consistent between sessions suggesting no effect of resistance exercise intensity on the complex transcriptional expression of the *PGC-1α* gene. IGF-1Ea mRNA expression significantly increased following the higher-intensity session compared with pre-exercise and the lower-intensity session. Myostatin mRNA expression was significantly reduced compared with pre-exercise values at all time points with no difference between exercise intensity. Further research is needed to determine the effects of the various isoforms of PGC-1α in human skeletal muscle on the translational level as well as their relation to the expression of IGF-1 and myostatin.

Key words: resistance exercise, gene expression, skeletal muscle, exercise intensity, PGC-1α, IGF-1, myostatin

Introduction

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a coactivator of transcription, first identified in brown adipose tissue (Puigserver et al. 1998). Since its discovery, PGC-1α has been demonstrated to facilitate a multitude of the beneficial adaptations in skeletal muscle induced by aerobic exercise such as mitochondrial biogenesis and enhanced capillarization (Holloszy et al. 1984; Puigserver et al. 2003). More recently, research investigating the role of PGC-1α has unveiled a very complex level of transcriptional regulation resulting in a multitude of PGC-1α mRNA isoforms (Martínez-Redondo et al. 2015). Transcription of the *PGC-1α* gene can initiate from an alternative promotor and/or undergo alternative splicing resulting in at least nine distinct mRNA transcripts and proteins. A detailed explanation of the known *PGC-1α* isoforms can be found in a review by Martinez-Redondo et al. (2015). Of the newly identified PGC-1α isoforms, Ruas et al. (2012) identified novel isoforms of PGC-1α that result from alternative promoter usage and splicing of the primary transcript generated from the *PGC-1α* gene. For identification purposes, the canonical PGC-1α isoform has been designated as PGC-1α1 (also named PGC-1α-a) and the novel PGC-1α isoforms identified by Ruas et al. as PGC-1α2, PGC-1α3, and PGC-1α4 (also named NT-PGC-1α-b; Ruas et al. 2012).

PGC-1α4, unlike other PGC-1α isoforms, has been implicated to have a role in skeletal muscle hypertrophy (Ruas et al. 2012). Mechanistically, PGC-1α4 expression is associated with changes in histone modifications near the IGF-1 and myostatin genes altering their transcription. Additionally, PGC-1α4 overexpression in mice resulted in robust skeletal muscle hypertrophy (Ruas et al. 2012). Furthermore, in mice subjected

3

to a hindlimb suspension/reloading protocol, PGC-1α4 expression reduced 22% during suspension and then increased substantially during reloading hypertrophy by 18.7-fold, accompanied by protein accumulation, increased IGF-1 gene expression, and decreased myostatin gene expression (Ruas et al. 2012). Data available in humans regarding PGC-1α4 mRNA expression shows a correlation with its expression and down-regulation of myostatin at 48 hours after both resistance exercise and combined resistance and endurance exercise training programs (Ruas et al., 2012). Additionally, PGC-1α4 expression was significantly correlated with leg press performance in these individuals after 8 weeks of training (Ruas et al., 2012). In contrast, the role of PGC-1α2 and PGC-1α3 within skeletal muscle is largely unknown. Because of the demonstrated evidence of a role for PGC-1α4 in skeletal muscle hypertrophy, more attention has been devoted to studying the mRNA expression of various PGC-1α isoforms in response to resistance exercise in humans.

In humans, PGC-1α splice variant expression has been demonstrated to increase as a result of resistance exercise (Ruas et al., 2012; Ydfors et al. 2013; Nader et al. 2014); however, any potential divergent effects of prominent resistance training variables, such as resistance exercise intensity, have yet to be explored. Therefore, the purpose of this study was to describe and compare the acute mRNA expression over 48 hours post-exercise for total PGC-1α and the PGC-1α splice variants in response to two different resistance exercise intensities in previously resistance-trained men using the real-time polymerase chain reaction (RT-PCR) primers described by Ruas et al. (2012). A secondary purpose of the study was to determine whether resistance exercise

4

intensity affects the post-exercise mRNA expression of genes potentially regulated by the PGC-1α4 isoform, including IGF-1Ea and myostatin.

Materials and methods

Participants

Ten apparently healthy, recreationally resistance-trained men (23.7 ± 2.8 years, 178.8 ± 5.9 cm, 85.9 ± 9.2 kg) volunteered to serve as participants in the study. Recreationally resistance-trained was defined as persons who resistance trained for general health and body composition purposes (i.e. 3 to 6 days per week for at least one year prior to the onset of the study), yet did not perform, with consistency, the volume of resistance training normally required in order to compete in professional strength or bodybuilding competitions or professionally competitive athletic events. The average continuous resistance training experience of the participants was 6.6 ± 2.4 years. Enrollment was open to men of all ethnicities. The current study was restricted to men in order to reduce any potential variability of gene expression due to sex-related differences. As the acute time course of the response of these particular novel PGC-1alpha isoforms were unknown, the current study was designed to focus on differences in resistance training intensity alone. Determining the optimal time points and loading parameters for which to study the expression of these particular genes will allow for better comparison of sex-specific differences in future studies. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM), and who

5

have not consumed any nutritional supplements (excluding multi-vitamins) three months prior to the study were allowed to participate. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Declaration of Helsinki.

Study Design

In a crossover, uniform-balanced design, recreationally resistance-trained men between the ages of 18 and 30 completed two lower-body resistance exercise sessions of different intensities (50% of one-repetition maximum (1-RM) and 80% of 1-RM) with equal volume-load. Each exercise session was separated by 7 to 10 days. Muscle samples from the vastus lateralis were acquired before exercise (PRE), 45 minutes post-exercise (45MINPE), 3 hours post-exercise (3HRPE), 24 hours post-exercise (24HRPE), and 48 hours post-exercise (48HRPE) to assess changes in gene expression of total PGC-1α and its respective isoforms, IGF-1Ea, and myostatin.

Familiarization Session

Participants expressing interest in participating in this study were interviewed on the phone or by e-mail to determine whether they qualified to participate in the study. Participants believed to meet eligibility criteria were then invited to attend an entry/familiarization session. Once reporting to the lab, participants completed a medical history questionnaire and underwent a general physical examination to determine whether they met eligibility criteria. Participants meeting entry criteria were

6

familiarized to the study protocol via a verbal and written explanation outlining the study design and signed the university-approved informed consent document. Next, assessments of body composition were performed. Total body mass (kg) was determined by using a calibrated electronic scale with a precision of ± 0.02 kg (Detecto, Webb City, MO). To sufficiently define the anthropometric characteristics of the sample population to be studied, percent body fat, fat mass, and fat-free mass were determined using dual energy x ray absorptiometry [(DEXA) Hologic Discovery, Bedford, MA]. After body composition assessment, one-repetition maximum (1-RM) assessments for resistance exercises (leg press and unilateral knee extension) to be performed during the testing sessions were completed. Participants warmed-up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM for each exercise. The participant rested for 1 minute, and then completed 3 to 5 repetitions at approximately 70% of the estimated 1-RM for each exercise. The weight was then increased conservatively, and the participant attempted to lift the weight for one repetition. If the lift was successful, the participant rested for 2 minutes before attempting the next weight increment. This procedure was continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for one repetition. Leg press strength was assessed using an isotonic hip/leg sled (Nebula Fitness, Inc., Versailles, OH). Knee extension strength was assessed using a modular isotonic leg extension machine (Cybex International, Inc., Medway, MA). Once the 1-RM of each leg was determined, participants were asked to perform and practice 1 or 2 sets with the proposed resistance for the exercise sessions without muscle

sampling to familiarize them with the protocol and to ensure they were able to complete the protocol before being formally admitted to the study.

At the conclusion of the familiarization session, participants were given an appointment in which to attend their first testing session. In addition, participants were instructed to refrain from exercise for at least 72 hours prior to the testing sessions, and to record their dietary intake for one day prior to and for the duration of each of the two testing sessions involved in the study.

Dietary Analyses

Participants were required to record their dietary intake for one day prior to and for the duration of each of the two testing sessions. The general diets of the participants were not standardized. To help standardize pre-workout food consumption, participants were asked to consume a protein bar (Premier Protein Bar, Premier Nutrition Corp., Emeryville, CA) one hour prior to each time point, with the exception of the 45 minute post-exercise time point for the which the bar was consumed immediately post-exercise allowing only 45 minutes for digestion and absorption. The dietary records were evaluated with the Food Processor dietary assessment software program (ESHA Research, Salem, OR) to determine the average daily kilocalorie and macronutrient consumption of fat, carbohydrate, and protein in the diet for the duration of the testing sessions.

Testing Sessions

8

Each participant performed two resistance exercise testing sessions of different intensities consisting of equal volume-load separated by 7 to 10 days. Each resistance exercise session consisted of the leg press using an isotonic hip/leg sled (Nebula Fitness) followed by unilateral isotonic knee extensions (Cybex International). The rest period between sets and exercises was standardized at 150 seconds in length. For the higher-intensity resistance exercise session, participants performed both exercises for 5 sets of 6 repetitions at 80% of 1-RM. For the lower-intensity resistance exercise session, participants performed both exercises for 3 sets of 16 repetitions at 50% of 1-RM. All repetitions were able to be completed at both testing sessions. Volume-load was calculated as sets x repetitions x weight.

Percutaneous muscle biopsies (15 to 20 mg) were obtained from the middle portion of the vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. The leg biopsed for the first testing session was chosen at random with the opposite leg being used for the second testing session. The biopsy area was shaved clean of leg hair, washed with antiseptic soap, and cleaned with rubbing alcohol. In addition, the biopsy site was further cleansed by swabbing the area with Betadine (fluid antiseptic). A small area of the cleaned skin approximately 2 cm in diameter was anesthetized with a 1.0 mL subcutaneous injection of the topical anesthetic Lidocaine. Once anesthetized, a 16-gauge fine needle aspiration biopsy instrument (Tru-Core I Biopsy Instrument, Medical Device Technologies, Gainesville, FL) was inserted into the skin at an approximate depth of 1 cm to extract the muscle sample. After removal, adipose tissue was trimmed from the muscle specimens. Specimens were immediately frozen in liquid nitrogen and

9

then stored at -80°C for later analysis. After the initial biopsy, the next biopsy attempts extracted tissue from approximately the same location (~1 cm away from the original site) as the initial biopsy by using the pre-biopsy markings and depth markings on the needle. Five muscle samples were obtained at each of the 2 testing sessions, with a total of 10 muscle samples being obtained during the course of the study. At each testing session, muscle samples were obtained immediately prior to the commencing the testing session (PRE), 45MINPE, 3HRPE, 24HRPE, and 48HRPE. Ruas et al. (2012) measured PGC-1α4 mRNA expression in humans prior to the onset of a resistance training program and 48 hours following the last resistance exercise session of the training program. The biopsy time points of the current study were chosen in order to describe the acute time course of PGC-1α isoform mRNA expression immediately following a resistance exercise bout leading up to the 48-hour time point described by Ruas et al.

Real-time polymerase chain reaction (PCR) to determine skeletal muscle mRNA expression

Approximately 10 - 15 mg of muscle tissue was used for biochemical analysis. Total cellular RNA was extracted from homogenate of biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO). The total RNA concentration was determined spectrophotometerically (SmartSpec Plus, Bio-Rad, Hercules, CA, USA) by optical density (OD) at 260 nm using an OD260 equivalent to 40 μg/μl and the final concentration expressed relative to muscle wet-weight. Aliquots of total RNA (5μl) were

10

separated with 1% agarose gel electrophoresis, ethidium bromide stained, and monitored under an ultraviolet light (Chemi-Doc XRS, Bio-Rad, Hercules, CA) to verify RNA integrity and absence of RNA degradation, indicated by prominent 28s and 18s ribosomal RNA bands, as well as an OD260/OD280 ratio of approximately 2.0. The RNA samples were stored at -80°C until later analysis. Five µg of total skeletal muscle RNA was reverse-transcribed to synthesize cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The cDNA concentration was determined by using an OD260 equivalent to 50 µg/µl and starting cDNA template concentration was standardized by adjusting all samples to 200 ng prior to amplification.

The mRNA sequences of human skeletal muscle IGF-1Ea (M37483) and myostatin (NM_005259), published in the NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov) were used to construct PCR primers using Beacon Designer software (Bio-Rad, Hercules, CA, USA), and then commercially synthesized (Integrated DNA Technologies, Coralville, IA). These primers amplify fragments of 150 base pairs (bp) for IGF-1Ea, and 145 bp for myostatin. The mRNA sequences of human skeletal muscle for total PGC-1α (recognizes all *PGC-1α* isoforms), PGC-1α1, PGC-1α2, PGC-1α3, and PGC-1α4 are not yet published in the NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov); therefore, PCR primers were constructed from the experimental procedures published by Ruas et al. (2012). However, using RT-PCR alone, these primers are not specific to each isoform. Subsequent to the original study by Ruas et al. (2012), it has been determined that the primer pairs described detect multiple isoforms using RT-PCR alone (Ydfors et al. 2013; Martínez-Redondo et al. 2015). The authors of the current study were unable to further elucidate the specific

11

expression of each isoform due to sample availability. Table 1 explains the primer sequences used for the current study and the additional isoforms detected by each primer pair. For brevity and consistency, the mRNA targets will still be referred to as total PGC-1α, PGC-1α1, PGC-1α2, PGC-1α3, and PGC-1α4.

Insert Table 1.

Using the primer sequences, PCR primers were constructed using Beacon Designer software (Bio-Rad, Hercules, CA, USA), and then commercially synthesized (Integrated DNA Technologies, Coralville, IA). Due to its consideration as a constitutively expressed "housekeeping gene," and the fact that it has been shown to be an appropriate external reference standard in human skeletal muscle using real-time PCR, β-actin (NM_001101) was used for detecting the relative change in the quantity of mRNA in response to resistance exercise (Mahoney et al. 2004). For β-actin, these primers amplify a PCR fragment of 135 base pairs. The relative expression of mRNA was determined from the β-actin/target CT ratio for each time point.

Statistical Analyses

Dependent t-tests were performed to determine differences for dietary intake between testing conditions. Statistical analyses of mRNA expression were performed by utilizing separate 2 x 5 (intensity x time point) two-way repeated-measures analyses of variance (ANOVA) for each criterion variable. Significant within-intensity and within-time differences were determined using Fisher's Least Significant Difference post-hoc

12

test. If within-group assumption of sphericity was violated using Mauchly's Test of Sphericity, the Greenhouse-Geisser correction factor was used to evaluate observed within-group F-ratios to protect against Type I error. Interaction effects were investigated using separate repeated-measures ANOVA for each intensity and time point. Effect size was measured using partial eta-squared (partial η^2). Based on the findings of pilot data within our lab, which determined PGC-1α mRNA expression to be significantly altered in response to resistance exercise (pre = 1.01 ± 0.03 arbitrary units (AUs), post = 1.09 ± 0.07 AUs), the sample size calculations for a two-tailed study design yielded a minimum sample size of 8 for each condition in order to attain a statistical power of 0.80. Therefore, to minimize the probability of making a type II error, 10 males were recruited for this study. All statistical procedures were performed using IBM SPSS Statistics 19.0 software and a probability level of < 0.05 was adopted. β-actin/target CT ratio data are presented as mean ± standard deviation (SD). Fold-changes from baseline were determined using the $2^{-(\Delta\Delta CT)}$ method (Livak et al. 2001).

Results

Detailed participant characteristics (body composition and relative strength) and dietary analyses data have been published previously (Schwarz et al. 2015). No differences in dietary analyses existed between testing sessions (p > 0.05).

Total PGC-1α mRNA Expression

The main effect of intensity revealed no statistically significant difference in total PGC-1α mRNA expression between trials [$F(1, 9) = 3.373$, $p = .099$, partial $\eta^2 = .273$].

The main effect of time demonstrated a statistically significant difference in total PGC-1α mRNA expression between time points [$F(4, 36) = 20.659$, $p < .001$, partial $\eta^2 = .697$]. There was no statistically significant interaction between intensity and time for total PGC-1α mRNA expression [$F(4, 36) = 1.951$, $p = .123$, partial $\eta^2 = .178$]. Post-hoc analyses revealed relative total PGC-1α mRNA expression was significantly reduced at 24HRPE ($p = .003$, fold change = 0.168) and 48HRPE ($p < .001$, fold-change = 0.175) compared with PRE. Data for total PGC-1α mRNA expression are shown in Figure 1.

Insert Figure 1 about here.

PGC-1α1 mRNA Expression

The main effect of intensity revealed no statistically significant difference in PGC-1α1 mRNA expression between trials [$F(1, 9) = .076$, $p = .790$, partial $\eta^2 = .008$]. The main effect of time demonstrated a statistically significant difference in PGC-1α1 mRNA expression between time points [$F(4, 36) = 6.286$, $p = .001$, partial $\eta^2 = .411$]. There was no statistically significant interaction between intensity and time for PGC-1α1 mRNA expression [$F(4, 36) = 2.596$, $p = .053$, partial $\eta^2 = .224$]. Post-hoc analyses revealed relative PGC-1α1 mRNA expression was significantly reduced at 24HRPE ($p = .004$, fold-change = 0.314) and 48HRPE ($p = .002$, fold-change = 0.267) compared with PRE. Data for PGC-1α1 mRNA expression are shown in Figure 2.

PGC-1α2 mRNA Expression

14

The main effect of intensity revealed no statistically significant difference in PGC-1α2 mRNA expression between trials [$F_{(1, 9)}$ = 4.489, p = .063, partial η^2 = .333]. The main effect of time demonstrated a statistically significant difference in PGC-1α2 mRNA expression between time points [$F_{(4, 36)}$ = 25.129, p < .001, partial η^2 = .736]. There was no statistically significant interaction between intensity and time for PGC-1α2 mRNA expression [$F_{(4, 36)}$ = 1.356, p = .268, partial η^2 = .131]. Post-hoc analyses revealed relative PGC-1α2 mRNA expression was significantly increased at 45MINPE (p = .004, fold-change = 24.675) and 3HRPE (p < .001, fold-change = 93.269) compared with PRE. Data for PGC-1α2 mRNA expression are shown in Figure 2.

PGC-1α3 mRNA Expression

The main effect of intensity revealed no statistically significant difference in PGC-1α3 mRNA expression between trials [$F_{(1, 9)}$ = 2.062, p = .185, partial $\eta2$ = .186] The main effect of time demonstrated a statistically significant difference in PGC-1α3 mRNA expression between time points [$F_{(4, 36)}$ = 5.563, p = .001, partial $\eta2$ = .382]. There was no statistically significant interaction between intensity and time for PGC-1α3 mRNA expression [$F_{(4, 36)}$ = .992, p = .424, partial $\eta2$ = .099]. Post-hoc analyses revealed PGC-1α3 mRNA expression significantly increased at 3HRPE (p = .002, fold-change = 6.498) and 48HRPE (p = .033, fold-change = 1.765) compared with PRE. Data for PGC-1α3 mRNA expression are shown in Figure 2.

PGC-1α4 mRNA Expression

15

The main effect of intensity revealed no statistically significant difference in PGC-1α4 mRNA expression between trials [$F(1, 9) = 1.766$, $p = .217$, partial $\eta^2 = .164$]. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for time [$\chi^2(9) = 18.383$, $p = .035$]; therefore, a Greenhouse-Geisser correction was applied ($\varepsilon = 0.583$). The main effect of time demonstrated a statistically significant difference in PGC-1α4 mRNA expression between time points [$F(2.33, 20.971) = 11.542$, $p < .001$, partial $\eta^2 = .562$]. There was no statistically significant interaction between intensity and time for PGC-1α4 mRNA expression [$F(4, 36) = 1.597$, $p = .196$, partial $\eta^2 = .151$]. Post-hoc analyses revealed PGC-1α4 mRNA expression to be significantly decreased at 45MINPE ($p = .007$, fold-change = 0.478) compared with PRE. At 3HRPE, PGC-1α4 mRNA expression significantly increased above expression at PRE ($p = .004$, fold-change = 2.761). At 48HRPE, PGC-1α4 mRNA expression significantly decreased below expression at PRE ($p = .018$, fold-change = 0.328). Data for PGC-1α4 mRNA expression are shown in Figure 2.

Insert Figure 2 about here.

IGF-1Ea mRNA Expression

The main effect of intensity revealed no statistically significant difference in IGF-1Ea mRNA expression between trials [$F(1, 9) = .506$, $p = .495$, partial $\eta^2 = .053$]. The main effect of time demonstrated no statistically significant difference in IGF-1Ea mRNA expression between time points [$F(4, 36) = .772$, $p = .551$, partial $\eta^2 = .079$]. However, a statistically significant interaction between intensity and time for IGF-1Ea mRNA

16

expression was observed [$F_{(4, 36)}$ = 2.929, p = .034, partial η^2 = .246]. Further analyses revealed no significant difference between time points on IGF-1Ea mRNA concentration for the 50% intensity exercise trial [$F_{(4, 36)}$ = .383, p = .819, partial η^2 = .041]. Conversely, a statistically significant difference for IGF-1Ea mRNA expression between time points existed for the 80% intensity exercise session [$F_{(4, 36)}$ = 2.801, p = .040, partial η^2 = .237]. Pairwise comparisons between time points for the 80% intensity exercise session revealed IGF-1Ea mRNA expression to be significantly higher at 24HRPE compared with PRE (p = .041, fold-change = 3.074). Repeated-measures ANOVA between intensities for each time point further revealed IGF-1Ea mRNA expression to be significantly higher at 24HRPE for the 80% intensity exercise session compared with the same time point for the 50% intensity exercise session [$F_{(1, 9)}$ = 12.831, p = .006, partial η^2 = .588]. Data for IGF-1Ea mRNA expression are shown in Figure 3.

Myostatin mRNA Expression

The main effect of intensity revealed no statistically significant difference in myostatin mRNA expression between trials [$F_{(1, 9)}$ = 3.994, p = .077, partial η^2 = .307]. The main effect of time demonstrated a statistically significant difference in myostatin mRNA expression between time points [$F_{(4, 36)}$ = 14.835, p < .001, partial η^2 = .622]. There was no statistically significant interaction between intensity and time for myostatin mRNA expression [$F_{(4, 36)}$ = 1.192, p = .331, partial η^2 = .117]. Post-hoc analyses revealed myostatin mRNA expression to be significantly decreased at all post-exercise time points compared with PRE (45MINPE, p = .001, fold-change = 0.277; 3HRPE, p =

17

.006, fold-change = 0.334; 24HRPE, $p < .001$, fold-change = 0.065; 48HRPE, $p < .001$, fold-change = 0.125). Data for myostatin mRNA expression are shown in Figure 3.

Insert Figure 3 about here.

Discussion

The current study demonstrated no difference in mRNA expression of PGC-1α isoforms in previously resistance-trained men as a result of resistance exercise intensity. Additionally, resistance exercise intensity did not differentially affect myostatin mRNA expression.   Conversely, IGF-1Ea mRNA expression was preferentially increased by higher-intensity resistance exercise in comparison with lower-intensity resistance exercise.

PGC-1α mRNA Expression

The *PGC-1α* primers used for this study were designed from the primer sequences published by Ruas et al. (2012). It is important to note that not all of the primers are solely specific to each PGC-1α isoform. Primer pair combinations needed to detect each isoform individually would result in amplicons too large to be ideal for use in RT-PCR. The various isoforms detected by each primer pair are already indicated in Table 1.

The current study was designed to fill gaps in the current literature regarding the temporal expression of PGC-1α splice variants in response to resistance exercise. At each time point, PGC-1α1 was the most abundantly expressed PGC-1α variant. As

18

more data become available, it is becoming evident that PGC-1α, which originates from the proximal promoter, is the least responsive splice variants to exercise stress. The results of Nader et al. (2014) and the current study observed no increase of PGC-1α1 compared with control or baseline values, respectively. In fact, both studies demonstrated a decrease in the PGC-1α1 isoform as a result of resistance exercise. Conversely, Ydfors et al. (2013) demonstrated an increase of PGC-1α1 mRNA expression in response to both endurance and resistance exercise. Besides the findings reported by Ydfors and colleagues, endurance exercise has consistently resulted in robust expression of PGC-1α isoforms originating from the alternative promoters (ex1b and ex1b') with little response from PGC-1α1 which is transcribed from the proximal promoter (Miura et al. 2008; Chinsomboon et al. 2009). However, the results of one study suggest that endurance exercise intensity is an important factor in PGC-1α1 expression, after demonstrating a change in its expression as a result of very high-intensity endurance exercise (Tadaishi et al. 2011). More studies are required to determine the exercise conditions required to stimulate robust transcriptional expression of PGC-1α1 to better elucidate its role in resistance exercise adaptations.

Whereas transcription of PGC-1α1 remained unresponsive and even decreased at 24HRPE and 48HRPE, PGC-1α2 was the most robustly expressed splice variant in response to resistance exercise. PGC-1α2 was negligibly expressed at baseline and quickly rose to levels comparable to PGC-1α1 at 3HRPE. Additionally, PGC-1α2 was the isoform combination most strongly induced by resistance exercise in the current study. PGC-1α3 mRNA expression responded to resistance exercise in a similar manner to PGC-1α2, but to a slightly lesser magnitude. The roles of PGC-1α2 and

19

PGC-1α3 in skeletal muscle adaptations as a result of exercise are largely unknown (Ruas et al. 2012; Martínez-Redondo et al. 2015). In the current study, these isoforms were responsive to resistance exercise. Additionally, Nader et al. (2014) observed that the acute expression of these isoforms in response to resistance exercise is enhanced in previously resistance-trained skeletal muscle versus untrained muscle. In agreement with the results of this study, other studies have observed PGC-1α2 and PGC-1α3 to be weakly expressed under resting conditions (Nader et al. 2014). These observations suggest these isoforms contribute little to the constitutive activity of the cell, but may play a large role in exercise adaptations. Cumulatively, evidence has demonstrated resistance exercise is a powerful stimulator of their transcription suggesting a potential role in skeletal muscle plasticity in response to resistance exercise (Ydfors et al. 2013; Nader et al. 2014).

Unlike PGC-1α2 and PGC-1α3, PGC-1α4 expression experienced an initial decrease at 45MINPE before peaking in expression at 3HRPE. After peaking at 3HRPE, expression of PGC-1α4 mRNA decreased to below baseline levels at 48HRPE. Direct comparison of studies investigating PGC-1α4 expression in response to exercise is particularly difficult because of major differences in design. Adding to this difficulty are the conflicting results reported. Consequently, the conditions stimulating PGC-1α4 expression, as well as the time course of its expression, after an acute bout of resistance exercise remain unclear. Ruas et al. (2012) reported PGC-1α4 mRNA expression to be significantly increased, compared with pre-training baseline expression, 48 hours after the last resistance exercise bout following an 8-week training protocol (found in response to both resistance training alone and combined endurance

20

and resistance training). In the current study, acute PGC-1α4 expression following a resistance exercise bout in resistance-trained individuals was triphasic: initially decreasing to below baseline levels at 45MINPE, increasing at 3HRPE, and then decreasing back to below baseline levels at 48HRPE. These results are surprising because they are in contrast to the increase in expression observed by Ruas et al. (2012) at the 48HRPE time point. One possible explanation for the contrasting observations is that Ruas et al. (2012) compared 48HRPE expression to pre-training baseline levels and not baseline expression prior to the final acute exercise bout in trained skeletal muscle. Therefore, it may be that successive acute resistance exercise bouts lead to an increased cumulative baseline expression of PGC-1α4 in resistance-trained skeletal muscle. However, this interpretation is problematic as expression of PGC-1α4 at 48HRPE in the current study was decreased compared with baseline.

Nader et al. (2014) observed greater expression of PGC-1α4 four hours after an acute resistance exercise bout in previously resistance-trained limbs versus non-resistance-trained limbs subjected to the same exercise bout. These results suggest an enhanced response of PGC-1α4 expression in previously resistance-trained limbs to an acute bout of resistance exercise. However, post-exercise expression was compared to that of control limbs limiting the temporal interpretation of these results. In comparison, Ydfors et al. (2013) observed an increase in PGC-1α4 mRNA two hours after both acute endurance and resistance exercise in previously untrained participants. In the current study, PGC-1α4 expression was induced at 3HRPE in response to both resistance exercise bouts. Unfortunately, the study by Ydfors et al. nor the current study compared temporal expression with previously trained or previously untrained individuals,

21

respectively. Thus, direct comparison of temporal responses of this isoform to acute exercise between groups of trained and untrained individuals is needed to accurately determine the effect of training status on its expression.

It is important to point out that, despite the changes in splice variant expression, total PGC-1α expression remained stagnant and then decreased following resistance exercise. This finding stresses the importance of measuring the expression of specific splice variants of *PGC-1α* in response to exercise. Given that specific isoforms were expressed in response to exercise stress while others were not, the dynamics of PGC-1α mRNA expression can be missed if the various isoforms are overlooked.

IGF-1Ea mRNA Expression

IGF-1Ea expression was affected differentially depending on the intensity of resistance exercise performed. At 24HRPE, IGF-1Ea expression was greater for the 80% intensity exercise session compared with baseline expression and expression at the same time point following the 50% intensity exercise bout. In contrast to the current findings, an earlier study investigating the effects of differing resistance exercise intensities found no difference in IGF-1Ea expression attributable to exercise intensity (Wilborn et al. 2009). A few key differences exist between the current study and the study by Wilborn and colleagues (2009) that may explain the dissimilar results. The current study included time points at 24HRPE and 48HRPE, and it was at these time points where the effect of intensity was observed. The last time point observed by Wilborn and associates was 6 hours post-exercise, making it possible an unobserved difference in expression occurred later in the post-exercise period. Another difference

22

between the studies was the volume load for each session. In the current study, the volume load for each exercise bout was identical, whereas in the other study volume load was significantly greater for the lower-intensity exercise bout. Therefore, it could be possible that difference in mRNA expression of IGF-1Ea as a result of exercise intensity are observed if volume load is equal, but that increasing volume of a lower-intensity protocol negates any differences based on intensity alone. Additionally, exercise intensities for the current study were 50% and 80% of 1-RM differing from the 60 to 65% and 80 to 85% of 1-RM employed by Wilborn and colleagues; therefore, the difference between intensities for their study may not have been large enough to elicit a differential response.

Interestingly, Wilborn et al. (2009) observed an increase in IGF-1Ea at 2-hr PE whereas changes were not seen in the current study until at least the 24HRPE time point. The difference in these results, at least for IGF-1Ea, may partially be explained by the training status of the participants for each study. In the current study, the participants were previously resistance-trained men whereas participants of the Wilborn et al. study were untrained. Most studies quantifying acute expression of IGF-1Ea in response to resistance exercise using untrained individuals observe no change in IGF-1Ea expression (Hameed et al. 2003; Petrella et al. 2006; Bamman et al. 2007; Hameed et al. 2008; Aperghis et al. 2009; Roberts et al. 2010). Some studies of untrained individuals do report an increase in IGF-1Ea mRNA at various time points post-exercise, yet when expression is observed it does not appear to follow a consistent pattern (Bamman et al. 2001; Greig et al. 2006; McKay et al. 2008; Wilborn et al. 2009). Conversely, and in agreement to the results of the current study, a consistent up-

23

regulation of IGF-1Ea mRNA expression is observed 24 hours to 48 hours following a resistance exercise bout in resistance-trained individuals as reported by multiple trials (Hameed et al. 2003; Petrella et al. 2006; Bamman et al. 2007; Ahtiainen et al. 2011).

PGC-1α4 mRNA expression was similar in response to both exercise intensities. If PGC-1α4 protein synthesis patterns follow the same pattern of its mRNA expression after resistance exercise, then its expression would not explain the divergent responses of IGF-1Ea expression between the two exercise intensities.   However, translational regulation of protein synthesis is highly complex and transcriptional rate and/or mRNA content does not necessarily reflect protein content. Ruas et al. (2012) implicated PGC-1α4 as a putative regulator of IGF-1 expression in skeletal muscle, and the results of the current study do not refute this finding. Yet, the results of the current study suggest that acute transcriptional regulation of PGC-1α4 in response to resistance exercise is not directly linked to transcription of IGF-1Ea.   Alternatively, divergent post-translational modifications to PGC-1α4 as a result of different resistance exercise intensities may potentially regulate the involvement of PGC-1α4 in IGF-1Ea expression. Such changes in protein activity would not be detected using the current study design. Research investigating direct PGC-1α4 protein involvement in the transcriptional regulation of IGF-1 in response to resistance exercise in humans is needed to elucidate the importance of PGC-1α4 in this regard.

Myostatin mRNA Expression

Myostatin mRNA expression was diminished at all time points following exercise with no differences attributable to exercise intensity.   Previous studies assessing

24

myostatin mRNA expression suggest that certain resistance exercise intensity must be met in order to reduce myostatin expression, with resistance exercise above that "intensity threshold" not resulting in differing myostatin expression (Wilborn et al. 2009; Laurentino et al. 2012; Agergaard et al. 2013). Observations of myostatin expression in response to two separate resistance exercise protocols with intensity workloads of 16% and 70% of 1-RM resulted in an attenuation of myostatin for only the 70% intensity condition (Agergaard, et al. 2013). Likewise, after resistance exercise with 20% or 80% of 1-RM, only the 80% intensity was capable of attenuating myostatin mRNA expression (Laurentino, et al. 2012). However, a specific exception is low-intensity resistance exercise under conditions of blood flow restriction. At 20% of 1-RM with blood flow restriction state; myostatin expression was attenuated more so than in response to an 80% intensity session (Laurentino, et al. 2012). Wilborn and colleagues (2009) observed that myostatin expression was reduced equally at 6 hours post-exercise in response to resistance exercise at 60 to 65% and 80 to 85% of 1-RM despite differences in volume load. However, it is also possible that the intensities were too similar to stimulate a differential response. The finding of no difference in expression between intensities for the current study are in agreement with those of Wilborn et al., even with equal volume load and an approximately 10% greater difference in resistance exercise intensity between sessions. Further research is warranted to decipher approximately what "intensity threshold" must be met in order to attenuate myostatin gene expression, if such a threshold exists.

Results of many previous studies observing the dynamics of myostatin mRNA expression suggest that resistance exercise training enhances the decrease in mRNA

25

expression of myostatin in response to an acute resistance exercise bout (Hulmi et al. 2007; Kim et al. 2007; Laurentino et al. 2012; Lundberg et al. 2013); although, not all studies agree (Willoughby 2004). The results of the current study may lend more evidence to this scenario; however, this statement is made with caution as the results of the current study lack a direct comparison between resistance-trained and non-resistance-trained individuals. Ruas et al. (2012) demonstrated that PGC-1α4 contains a distinct N-terminal sequence important in allowing the protein to accumulate in skeletal muscle tissue. Additionally, PGC-1α4 overexpression is associated with repressive epigenetic modifications in proximity of the myostatin gene. Resistance exercise training may result in an accumulation of PGC-1α4 protein making previously trained skeletal muscle capable of diminishing myostatin mRNA expression to a much greater degree than in untrained skeletal muscle.

Limitations of the Study

The most notable limitation of the current study was the inability to detect the specific PGC-1α mRNA isoforms because of the use of RT-PCR alone. Additionally, the current study did not investigate PGC-1α activity on the translational level, limiting speculation as to its involvement in the expression of IGF-1Ea and myostatin. A lack of mRNA expression from a control limb also limits the insight into the variability of gene expression that may have been associated with inflammation of the biopsy procedure itself. Lastly, the current study was acute in nature and does not represent changes after chronic resistance exercise.

26

Conclusion

In conclusion, resistance exercise enhances the expression of the *PGC-1α* gene, primarily from the alternative promoter. The dynamics of PGC-1α mRNA expression is altered despite a lack of increase in total PGC-1α mRNA. Myostatin mRNA expression is reduced up to 48 hours following resistance exercise without differential influence by resistance exercise intensities of 50% of 1-RM and 80% of 1-RM. Additionally, higher-intensity resistance exercise results in greater acute expression of IGF-1Ea when compared to lower-intensity resistance exercise. Future study is needed to determine the importance of these findings at the translational level in response to chronic resistance exercise.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

References

Agergaard, J., Reitelseder, S., Pedersen, T.G., Doessing, S., Schjerling, P., Langberg, H., et al. 2013. Myogenic, matrix, and growth factor mRNA expression in human skeletal muscle: Effect of contraction intensity and feeding. Muscle & Nerve, **47**: 748-759. doi:10.1002/mus.23667.

Ahtiainen, J.P., Lehti, M., Hulmi, J.J., Kraemer, W.J., Alen, M., Nyman, K., et al. 2011. Recovery after heavy resistance exercise and skeletal muscle androgen receptor and insulin-like growth factor-I isoform expression in strength trained men. J Strength Cond Res. **25**: 767-777. doi:10.1519/JSC.0b013e318202e449.

Aperghis, M., Velloso, C.P., Hameed, M., Brothwood, T., Bradley, L., Bouloux, P.M., et al. 2009. Serum IGF-I levels and IGF-I gene splicing in muscle of healthy young males receiving rhGH. Growth Horm IGF Res. **19**: 61-67. doi:10.1016/j.ghir.2008.07.002.

Bamman, M.M., Petrella, J.K., Kim, J.S., Mayhew, D.L., and Cross, J.M. 2007. Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. J Appl Physiol. **102**: 2232-2239. doi:10.1152/japplphysiol.00024.2007.

Bamman, M.M., Shipp, J.R., Jiang, J., Gower, B.A., Hunter, G.R., Goodman, A., et al. 2001. Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. Am J Physiol Endocrinol Metab. **280**: E383-390. PMID:11171591.

Chinsomboon, J., Ruas, J., Gupta, R.K., Thom, R., Shoag, J., Rowe, G.C., et al. 2009. The transcriptional coactivator PGC-1α mediates exercise-induced angiogenesis in skeletal muscle. Science Signaling, **106**: 21401-21406. doi:10.1073/pnas.0909131106.

Greig, C.A., Hameed, M., Young, A., Goldspink, G., and Noble, B. 2006. Skeletal muscle IGF-I isoform expression in healthy women after isometric exercise. Growth Horm IGF Res. **16**: 373-376. doi:10.1016/j.ghir.2006.09.005.

Hameed, M., Orrell, R.W., Cobbold, M., Goldspink, G., and Harridge, S.D. 2003. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol. **547**: 247-254. doi:10.1111/j.1469-7793.2003.00247.x.

Hameed, M., Toft, A.D., Pedersen, B.K., Harridge, S.D., and Goldspink, G. 2008. Effects of eccentric cycling exercise on IGF-I splice variant expression in the muscles of young and elderly people. Scand J Med Sci Sports. **18**: 447-452. doi:10.1111/j.1600-0838.2007.00714.x.

Holloszy, J.O. and Coyle, E.F. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol. **56**: 831-838. PMID:6373687.

Hulmi, J.J., Ahtiainen, J.P., Kaasalainen, T., Pollanen, E., Hakkinen, K., Alen, M., et al. 2007. Postexercise myostatin and activin IIb mRNA levels: effects of strength training. Med Sci Sports Exerc. **39**: 289-297. doi:10.1249/01.mss.0000241650.15006.6e.

28

Kim, J.S., Petrella, J.K., Cross, J.M., and Bamman, M.M. 2007. Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis. J Appl Physiol. **103**: 1488-1495. doi:10.1152/japplphysiol.01194.2006.

Laurentino, G.C., Ugrinowitsch, C., Roschel, H., Aoki, M.S., Soares, A.G., Neves, M., et al. 2012. Strength training with blood flow restriction diminishes myostatin gene expression. Med Sci Sports Exerc. **44**: 406-412. doi:10.1249/MSS.0b013e318233b4bc.

Livak, K.J. and Schmittgen, T.D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods, **25**: 402-408. doi:10.1006/meth.2001.1262.

Lundberg, T.R., Fernandez-Gonzalo, R., Gustafsson, T., and Tesch, P.A. 2013. Aerobic exercise does not compromise muscle hypertrophy response to short-term resistance training. J Appl Physiol. **114**: 81-89. doi:10.1152/japplphysiol.01013.2012.

Mahoney, D.J., Carey, K., Fu, M.H., Snow, R., Cameron-Smith, D., Parise, G., et al. 2004. Real-time RT-PCR analysis of housekeeping genes in human skeletal muscle following acute exercise. Physiol Genomics. **18**: 226-231. doi:10.1152/physiolgenomics.00067.2004

Martínez-Redondo, V., Pettersson, A., and Ruas, J. 2015. The hitchhiker's guide to PGC-1α isoform structure and biological functions. Diabetologia, **58**: 1969-1977. doi:10.1007/s00125-015-3671-z

McKay, B.R., O'Reilly, C.E., Phillips, S.M., Tarnopolsky, M.A., and Parise, G. 2008. Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging muscle-lengthening contractions in humans. J Physiol. **586**: 5549-5560. doi:10.1113/jphysiol.2008.160176.

Miura, S., Kai, Y., Kamei, Y., and Ezaki, O. 2008. Isoform-specific increases in murine skeletal muscle peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA in response to β2-adrenergic receptor activation and exercise. Endocrinology, **149**: 4527-4533. doi:http://dx.doi.org/10.1210/en.2008-0466.

Nader, G.A., von Walden, F., Liu, C., Lindvall, J., Gutmann, L., Pistilli, E.E., et al. 2014. Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy. J Appl Physiol. **116**: 693-702 (1985). doi:10.1152/japplphysiol.01366.2013.

Petrella, J.K., Kim, J.S., Cross, J.M., Kosek, D.J., and Bamman, M.M. 2006. Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. Am J Physiol Endocrinol Metab. **291**: E937-946. doi:10.1152/ajpendo.00190.2006.

Puigserver, P. and Spiegelman, B.M. 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α): transcriptional coactivator and metabolic regulator. Endocr Rev. **24**: 78-90. doi:http://dx.doi.org/10.1210/er.2002-0012.

Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M., and Spiegelman, B.M. 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell, **92**: 829-839. doi:10.1016/S0092-8674(00)81410-5.

Roberts, M.D., Dalbo, V.J., Sunderland, K.L., Poole, C.N., Hassell, S.E., Bemben, D., et al. 2010. IGF-1 splice variant and IGF-1 peptide expression patterns in young and old human skeletal muscle prior to and following sequential exercise bouts. Eur J Appl Physiol. **110**: 961-969. doi:10.1007/s00421-010-1588-2.

Ruas, J.L., White, J.P., Rao, R.R., Kleiner, S., Brannan, K.T., Harrison, B.C., et al. 2012. A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell, **151**: 1319-1331. doi:10.1016/j.cell.2012.10.050.

Schwarz, N.A., Spillane, M.B., McKinley, S.K., Andre, T.L., Gann, J.J., and Willoughby, D.S. 2015. Acute Myosin Heavy Chain Isoform mRNA Expression in Response to Two Resistance Exercise Intensities with Equal Volume Load in Resistance-Trained Men. J Strength Cond Res. **29**: 2326-2332. doi:10.1519/JSC.0000000000000878.

Tadaishi, M., Miura, S., Kai, Y., Kawasaki, E., Koshinaka, K., Kawanaka, K., et al. 2011. Effect of exercise intensity and AICAR on isoform-specific expressions of murine skeletal muscle PGC-1alpha mRNA: a role of beta(2)-adrenergic receptor activation. Am J Physiol Endocrinol Metab. **300**: E341-349. doi:10.1152/ajpendo.00400.2010.

Wilborn, C.D., Taylor, L.W., Greenwood, M., Kreider, R.B., and Willoughby, D.S. 2009. Effects of different intensities of resistance exercise on regulators of myogenesis. J Strength Cond Res. **23**: 2179-2187. doi:10.1519/JSC.0b013e3181bab493.

Willoughby, D.S. 2004. Effects of heavy resistance training on myostatin mRNA and protein expression. Med Sci Sports Exerc. **36**: 574-582. doi:10.1249/01.MSS.0000121952.71533.EA.

Ydfors, M., Fischer, H., Mascher, H., Blomstrand, E., Norrbom, J., and Gustafsson, T. 2013. The truncated splice variants, NT-PGC-1alpha and PGC-1alpha4, increase with both endurance and resistance exercise in human skeletal muscle. Physiol Rep. **1**: e00140. doi:10.1002/phy2.140.

30

Table 1. Real Time-Polymerase Chain Reaction (RT-PCR) primer sequences for the targeted PGC-1α isoforms and the additional isoforms detected by each.

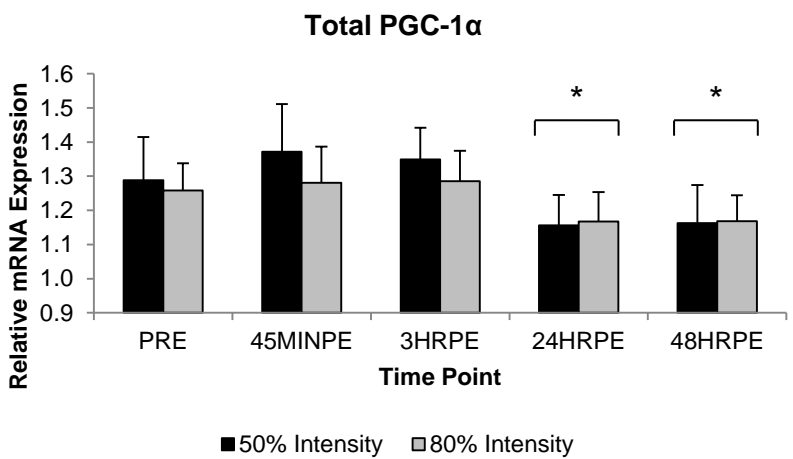| Target	Also Detected	Primer sequences
Total PGC-1α	N/A	Forward: 5'-CAGCCTCTTTGCCCAGATCTT-3' Reverse: 5'-TCACTGCACCACTTGAGTCCAC-3'
PGC-1α1/PGC-1α-a	NT-PGC-1α-a	Forward: 5'-ATGGAGTGACATCGAGTGTGCT-3' Reverse: 5'-GAGTCCACCCAGAAAGCTGT-3'
PGC-1α2	PGC-1α4 PGC-1α-b	Forward: 5'-AGTCCACCCAGAAAGCTGTCT-3' Reverse: 5'-ATGAATGACACACATGTTGGG-3'
PGC-1α3	PGC-1α-c NT-PGC-1α-c	Forward: 5'-CTGCACCTAGGAGGCTTTATGC-3' Reverse: 5'-CAATCCACCCAGAAAGCTGTCT-3'
PGC-1α4/NT-PGC-1α-b	NT-PGC-1α-a NT-PGC-1α-c	Forward: 5'-TCACACCAAACCCACAGAGA-3' Reverse: 5'-CTGGAAGATATGGCACAT-3'

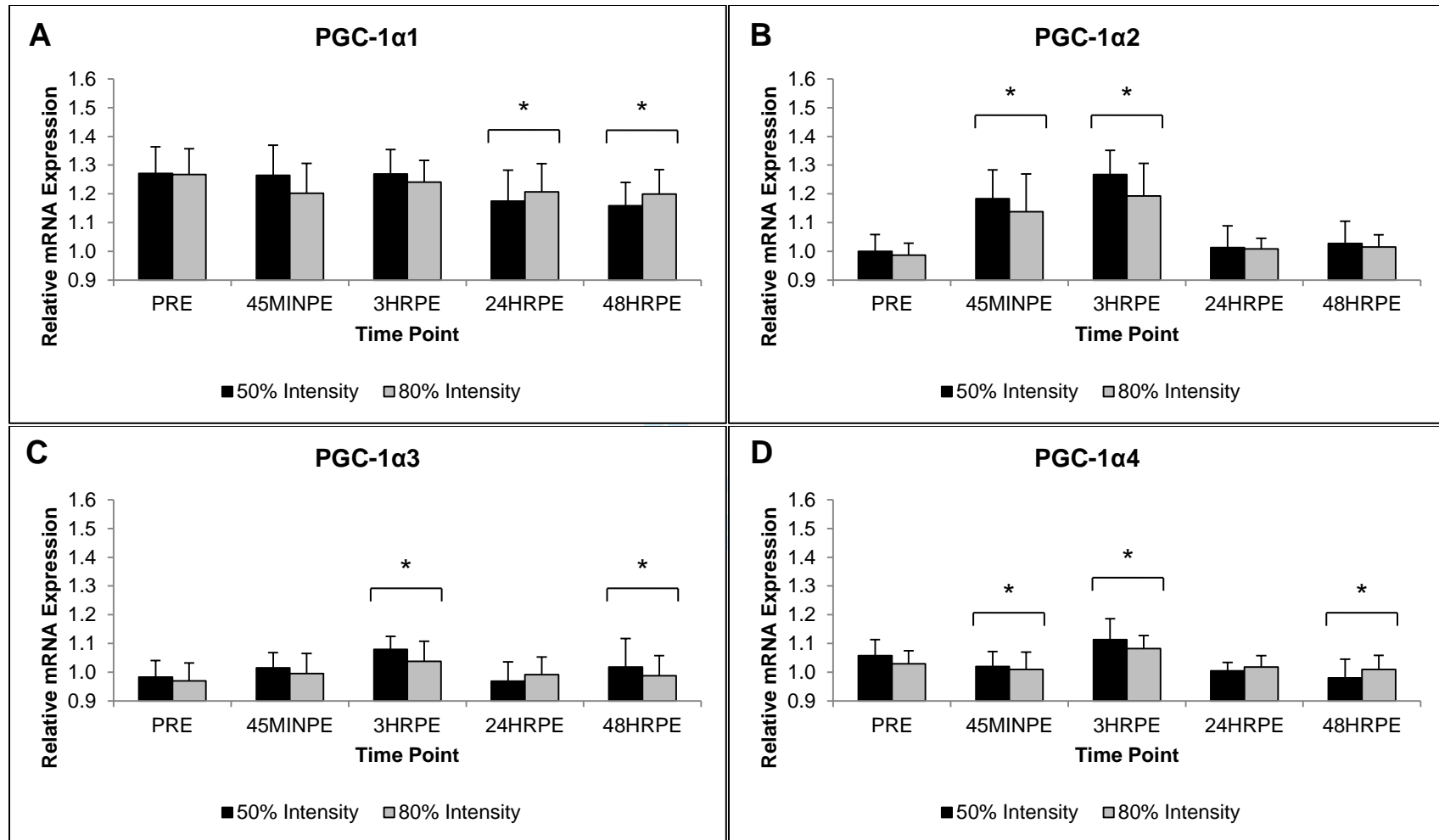**Note:** PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; NT, N-truncated; N/A, not applicable.

31

Fig. 1. Total peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) mRNA expression for each exercise intensity (50% or 80% of 1-repetition maximum (RM)) before exercise (PRE) and at 45 minutes (45MINPE), 3 hours (3HRPE), 24 hours (24HRPE), and 48 hours (48HRPE) post-exercise.   The values are presented as means ± SD, and the results are expressed relative to β-actin. Asterisk (*) indicates that the difference in relation to PRE was significant at $p < 0.05$.
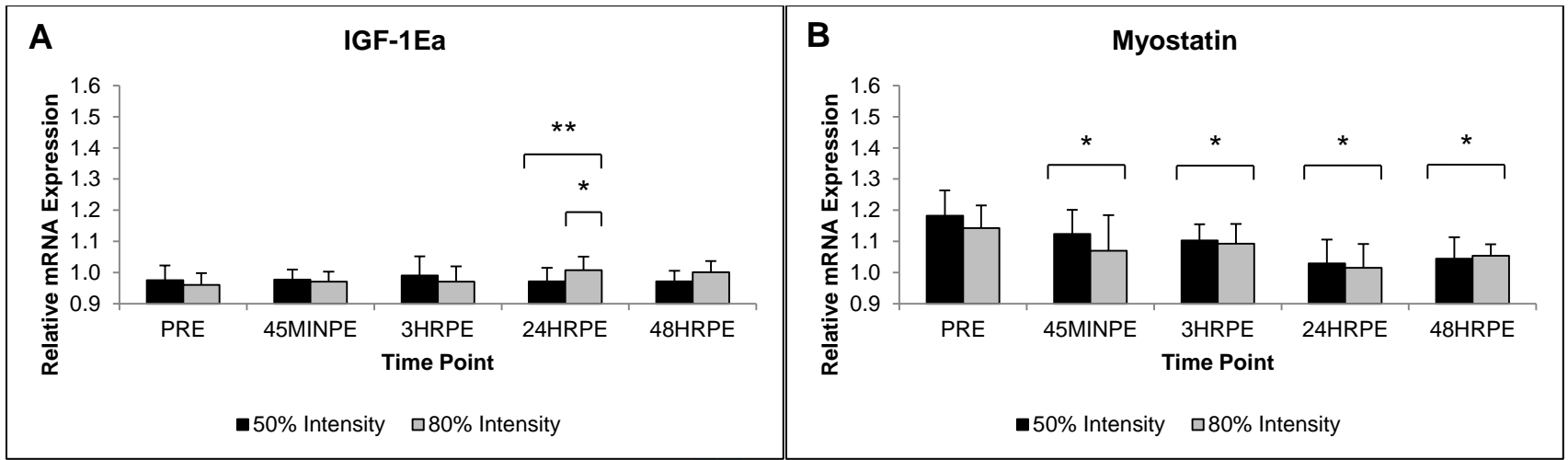
Fig. 2. Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) mRNA expression of each isoform (A, PGC-1α1; B, PGC-1α2; C, PGC-1α3; D, PGC-1α4) for each exercise intensity (50% or 80% of 1-repetition maximum (RM)) before exercise (PRE) and at 45 minutes (45MINPE), 3 hours (3HRPE), 24 hours (24HRPE), and 48 hours (48HRPE) post-exercise.   The values are presented as means ± SD, and the results are expressed relative to β-actin. Asterisk (*) indicates that the difference in relation to PRE was significant at $p < 0.05$.

Fig. 3. Insulin-like growth factor-1Ea (A, IGF-1Ea) and myostatin (B) mRNA expression for each exercise intensity (50% or 80% of 1-repetition maximum (RM)) before exercise (PRE) and at 45 minutes (45MINPE), 3 hours (3HRPE), 24 hours (24HRPE), and 48 hours (48HRPE) post-exercise.   The values are presented as means ± SD, and the results are expressed relative to β-actin. Asterisk (*) indicates that the difference in relation to PRE was significant at $p < 0.05$. Double asterisk (**) indicates that the difference between exercise intensities at the specified time point was significant at $p < 0.05$.

Total PGC-1α

A IGF-1Ea

B Myostatin