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Effect of Periodized Resistance Training on Skeletal Muscle During Androgen Deprivation Therapy for Prostate Cancer: A Pilot Randomized Trial

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Abstract

Purpose: Prostate cancer survivors (PCS) receive androgen deprivation therapy (ADT) as treatment for recurrent cancer, yet ADT is associated with loss of skeletal muscle and physical function. Resistance training can counter both muscle and physical function loss; however, an understanding of the molecular responses of skeletal muscle to resistance training during ADT is still undefined. This sub-analysis of the original randomized, controlled pilot trial investigated effects of 12 weeks of periodized resistance training on mRNA expression of the anabolic genes IGF-1, myogenin, PGC-1α4 and the catabolic genes myostatin and MuRF-1 in skeletal muscle of PCS on ADT. Secondary aims investigated if changes in lean mass and physical function correlated with changes in mRNA expression. **Methods:** PCS on ADT (n = 17) were randomized to 12 weeks of supervised resistance training (EXE, n = 9) or home-based stretching (STRETCH, n = 8) 3 days per week. Outcomes were assessed at baseline and post-intervention. Muscle biopsies were analyzed by RT-PCR for mRNA expression. Body composition was assessed through dual-energy X-ray absorptiometry, and physical function through muscular strength, timed up and go, stair climb, and 400 m walk. **Results:** MuRF-1 mRNA expression was significantly greater in EXE compared to STRETCH post-intervention ($P = .005$). Change in MuRF-1 mRNA expression significantly correlated with improvements in strength and physical function ($P < .05$), while change in IGF-1 expression correlated with change in lean mass ($P = .015$). **Conclusion:** Twelve weeks of resistance training increased mRNA expression of MuRF-1 in skeletal muscle of PCS on ADT. Elevations in resting mRNA expression of IGF-1, myogenin and PGC-1α4, and reduction in mRNA expression of myostatin that are typically expected following resistance training were not observed.

Keywords

strength training, prostate cancer, gene expression, sarcopenia

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Introduction

In men with locally advanced or metastatic prostate cancer, treatment with androgen deprivation therapy (ADT) confers substantial benefit by deferring symptom progression and increasing survival, especially when combined with radiation therapy.[1] However, ADT is associated with a loss of skeletal muscle that can impact physical well-being and increase risk of metabolic disease.[2] Resistance training is an established countermeasure to ADT-related reductions in skeletal muscle and physical function,[3] but only a few investigations have examined the effect of exercise on cellular responses underpinning skeletal muscle changes in prostate cancer survivors (PCS) on ADT.[4-7]

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In investigating the iatrogenic effects of ADT on skeletal muscle quality, Lamboley et al[5] found reduced contractile performance of skeletal muscle fibers in PCS on ADT compared to age-matched controls. Hanson et al[4] observed blunted muscle protein synthesis after protein ingestion in PCS on ADT compared to age-matched control subjects, which was rescued when protein ingestion was combined with resistance exercise. Nilsen et al[6,7] investigated skeletal muscle cellular outcomes after 16 weeks of resistance training in PCS on ADT, but only observed significant changes muscle cross sectional area and strength. Collectively, these studies support a role of resistance exercise in stimulating muscle protein synthesis and improving muscle quality during ADT, but an understanding of the molecular targets involved in skeletal muscle remodeling due to training in PCS is still limited.

The molecular pathways mediating the pro-atrophy skeletal muscle remodeling during ADT has been previously summarized.[8] Activation of genes that regulate proteolytic pathways, such as myostatin and the muscle-specific E3 ubiquitin ligase muscle ring finger 1 (MuRF-1), result in protein degradation,[8] yet are decreased following acute and chronic resistance exercise in healthy adults.[9-11] Also responsive to exercise are the myogenic factor myogenin and insulin-like growth factor-1 (IGF-1), the Akt/mammalian target of rapamycin (mTOR) pathway, where elevations in mRNA expression have been observed in healthy adults after acute and chronic resistance exercise.[12-14] In addition, a splice variant of the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) known as PGC-1α4, has been shown to affect both catabolic and anabolic pathways.[15] Preferentially induced during resistance exercise, PGC-1α4 inhibited transcriptional activity of the ubiquitin-proteasome system and promoted muscle hypertrophy.[15]

As mRNA expression of these molecular markers has not yet been assessed in PCS on ADT, an understanding of the molecular targets involved in skeletal muscle protein synthesis and breakdown may contribute to strategies mitigating the adverse effects of ADT. Therefore, the primary aim of this study was to investigate the effect of 12 weeks of resistance training on mRNA expression of anabolic regulators of skeletal muscle, including IGF-1, myogenin and PGC-1α4, and catabolic regulators of skeletal muscle, including myostatin and MuRF-1, in PCS on ADT. Secondary aims investigated correlations between changes in mRNA expression and changes in skeletal muscle mass and physical function. This exploratory study was conducted as part of a larger pilot trial, in which 12 weeks of resistance training was found to significantly increase muscle mass, strength and quality of life in PCS on ADT.[16] We hypothesized that 12 weeks of resistance training would elevate anabolic mRNA expression and reduce catabolic mRNA expression in PCS on ADT.

Materials and Methods

A detailed description of the study protocol has been previously described[17] and findings from the parent trial on body composition, metabolic syndrome, and physical function have been published.[16]

Recruitment and Randomization

Participants were recruited from hospitals in the Los Angeles area between May 2014 and March 2017 by physicians, nurses and physical therapists, and through newspaper advertisements. Eligible participants were diagnosed with prostate cancer, aged 50 or older, free of contraindications to exercise and currently treated with gonadotrophin-releasing hormone (GnRH) agonist/antagonist with or without anti-androgen for at least 12 weeks. Exclusion criteria included chemotherapy or radiation therapy within 4 weeks of enrollment, major surgery within the previous 6 months, acute coronary or vascular event in the last year, or current participation in a structured exercise program. Metastatic patients were not excluded. The randomization list was prepared in advance by the biostatistician and provided to the Clinical Investigation Support Office (CISO) at the USC Norris Comprehensive Cancer Center. To prevent potential bias, study personnel did not have access to the randomization list. Trial participants and investigators were not blinded to the group assignment. In the parent study, patients were randomly allocated (1:1:1:1) by CISO to 12 weeks of (1) resistance training + protein supplementation, (2) resistance training, (3) protein supplementation, or (4) control stretching. As small effect sizes were found between the 4 groups for all endpoints, the 4-arm design was collapsed into 2 groups of either EXE (received resistance training) or STRETCH (no resistance training). In the present study, EXE consisted of 4 participants who received resistance training + protein supplementation and 4 participants who received resistance training only, while STRETCH consisted of 4 participants who received protein supplementation and 3 participants who received control stretching. The study was approved by the University of Southern California (USC) Institutional Review Board (HS-13-00315) and all participants provided written informed consent.

Interventions

Resistance training program (EXE). EXE performed one-on-one resistance training 3 days per week for 12 weeks with a certified exercise trainer at the USC Clinical Exercise Research Center. The resistance training program met the American College of Sports Medicine/American Cancer Society's recommendation for strength training[18] and has been previously described in detail.[17] Briefly, each ~50 minutes session began with a 5 minutes dynamic warmup. The

training routine included 7 upper and lower extremity machine-based exercises and 3 trunk exercises. Weekly training volume was divided such that lower extremities/trunk musculature were trained on Day 1, lower and upper extremities trained on Day 2, and upper extremities/trunk musculature trained on Day 3. Every session concluded with 5 minutes of static stretching targeting the muscle groups used in the routine. The resistance training program was progressed over the course of the intervention using a periodization model consistent with a hypertrophy training goal for the majority of the program. Load assignments were calculated from estimated 1 repetition maximum (RM) values, which were determined from 10RM tests at baseline and at the end of week 6. The progression cycle was as follows: Weeks 1 to 2: 60% 1RM, 15 repetitions; Weeks 3 to 4: 65% to 67% 1RM, 15 to 12 repetitions; Weeks 5 to 6: 70% 1RM, 12 to 10 repetitions; Weeks 7 to 8: 75% 1RM, 10 to 8 repetitions; Weeks 9 to 10: 80% 1RM, 10 to 8 repetitions; Weeks 11 to 12: 83% 1RM, 8 repetitions. Rest periods were timed at exactly 1 minute between sets, and 3 sets of each exercise were performed. Participants were trained to fatigue, where light spotting and encouragement were provided for the last few reps of the third set when necessary.

Home-based stretching program (STRETCH). STRETCH performed a home-based flexibility program 3 times per week for 12 weeks, with each session lasting ~5 minutes. The stretching exercises in the home-based program matched those performed by EXE at the end of each training session. To increase compliance, STRETCH participants were given a stretching band and a booklet detailing the exercises. Participants completed weekly records of flexibility and self-directed exercises and were given a monetary compensation for the completed records. All STRETCH participants were offered the resistance training program after the post-intervention assessments.

Outcome Measures

All study outcomes were assessed at baseline and at the end of the 12-week intervention. An additional assessment of strength testing was performed at 6 weeks to update the %RM loads of the training program for weeks 7 to 12.

Anthropometric measurements. Anthropometric measures were obtained following a 12 hours fast and measured by the same experienced investigator. Whole-body and regional lean mass, and body fat percent were assessed through whole body dual-energy X-ray absorptiometry (DXA, Lunar GE iDXA, Fairfield, CT). Weight was measured on an electronic scale to the nearest 0.1 kg (InBody 570, InBody USA, Cerritos, CA) and height was measured on a standard stadiometer, with the value rounded to the nearest 0.5 cm.

Physical function. Physical function outcomes included the 400 m walk, Timed Up and Go, stair climb and muscular strength. For the 400 m walk, Timed Up and Go and stair climb, time was recorded to the nearest 0.1 second using a stopwatch. The Timed Up and Go and stair climb were performed in triplicate, and average score was reported. Maximal voluntary strength was assessed through 10RM tests at baseline, intervention midpoint (6 weeks) and post-intervention. Due to fragility concerns in individuals with bone metastatic disease, multiple-repetition testing was selected to minimize orthopedic issues that could arise from high force single repetition maximum testing.[19] In the parent trial, both upper and lower extremity exercises were tested, but this report focuses on the lower extremity exercises of leg press, leg curl, and leg extension. Prior to baseline testing, participants were instructed in proper technique over 2 familiarization sessions. During the testing session, participants completed a warm-up at a load of ~20RM, which was estimated based on investigator experience and verbal questions pertaining to training history. Three attempts were given to reach the final 10RM load with a 2 minutes rest period between attempts. Feedback on range of motion and verbal encouragement were given by the investigator after each repetition. To determine load assignments used in the periodization model, 1RM values were calculated from the 10RM load at baseline and 6 weeks using specific regression equations for leg press,[20] while all other exercises used 1RM = 10RM/0.75.[21]

mRNA expression. Exercise-responsive molecular endpoints were selected to reflect signaling of various targets along the anabolic and catabolic pathways.[9,11,15,22] Anabolic genes included IGF-1, PGC-1α, and myogenin, while catabolic genes included myostatin and MuRF-1.

Muscle biopsy. Muscle biopsies were performed as an optional procedure at the discretion of the participant before and after the intervention, with participants monetarily compensated for each biopsy. Specimens (25-30 mg) were collected from the vastus lateralis muscle midway between the patella and greater trochanter by a credentialed study physician. Under local anesthesia (1% lidocaine) and sterile conditions, an incision was made in the skin and fascia, and a 5-mm Stille muscle biopsy needle (Micrins Surgical, Lake Forest, IL) was used to obtain the specimen.[22,23] Samples were immediately flash-frozen in liquid nitrogen and stored at −80°C for later analysis. The incision was closed with a stitch, which was removed 48 hours after the biopsy. The biopsies occurred following a 12 hours fast and at the same time of day for baseline and post-intervention time points. The post-intervention biopsy was performed at a distance ~1 cm from the first incision and 72 to 96 hours after the last training session for EXE participants to reflect resting levels of mRNA expression.[24]

Table 1. Primers Used in Quantitative Real-Time PCR.

Gene	GenBank accession	Assay ID or reference	Product size (bp)
IGF-1	NM_000618	Hs.PT.58.21022358	136
GAPDH	NM_002046	Hs.PT.39a.22214836	143
PGC-1α	NM_013261	Ruas et al,[15] Zhang et al[26]	145
MuRF-1	NM_032588	Hs.PT.58.39092203	132
Myogenin	NM_002479	Hs.PT.58.38897870	124
Myostatin	NM_005259	Hs.PT.58.40523213	127

Total RNA isolation and cDNA synthesis. Muscle samples weighing approximately 20 mg were homogenized (Kinematica Polytron PT1200C) in TRIzol reagent (Zymo Research, Irvine, CA) prior to total RNA extraction and treatment with DNase I according to the manufacturer's instructions (DirectZol RNA MiniPrep Plus, Zymo Research, Irvine, CA). RNA concentration and purity were determined through spectrophotometry (NanoDrop ND-1000, Thermo Scientific, Waltham, MA) at an absorbance of 260 and 260 nm:280 nm ratio, respectively.[9-11] One participant's samples did not meet qualification standards for amount of RNA and was not included in further analyses. Reverse transcription was performed on 500 to 1000 ng total RNA (iCycler, Bio-Rad, Hercules, CA) with oligo hexamers in a total volume of 50 µL per manufacturer instructions (Taqman reverse transcriptase, Applied Biosystems, Foster City, CA).

Quantitative real-time PCR (qPCR). Relative levels of mRNA expression were determined using SYBR Green (PerfeC$_T$a SYBR Green SuperMix, Quanta Biosciences, Gaithersburg, MD) on an iCycler iQ system (Bio-Rad, Hercules, CA). Each 25 µL reaction contained 12.5 µL SYBR Green, 0.5 µL RNAse free water, 10 µL cDNA, and 1 µL of each primer (forward and reverse). Reactions were run in triplicate, with a non-template control included in each run. Both pre- and post-intervention samples from a given participant were run together, allowing the relative comparison of samples. Predesigned primer sequences were obtained from Integrated DNA Technologies, with the exception of PGC-1α4, which was designed using the Primer3 Plus program[25] to measure the expression of the N-truncated PGC-1α splice variant (NT-PGC-1α) by aligning to exons 5 and 7a.[15,26] Efficiencies of each primer set were tested using calibration curves, and the specificity of the amplification was checked through melt curve analysis. Gene accession numbers and assay IDs for the primer sets are included in Table 1. Average mRNA expression for each pre- and post-intervention sample was calculated from the 3 replicates. The $2^{-\Delta\Delta CT}$ method[27] was used to compute fold change of the relative changes in mRNA expression between EXE and STRETCH from pre- to post-intervention. Each gene of interest was normalized to the control gene, GAPDH, as previous investigations have shown no changes in GAPDH levels following resistance exercise in human skeletal muscle.[9]

Physical Activity and Dietary Assessments

Physical activity history was assessed at baseline using the International Physical Activity Questionnaire.[28] At baseline and every week during the intervention period, participants completed a 3-day dietary food intake. To increase compliance, participants were given a monetary compensation for each log. The dietary records were analyzed under the supervision of a registered dietician to ensure accuracy in portion size before being entered in an online nutritional analysis application (My Fitness Pal, Under Armour, myfitnesspal.com). Participants who did not meet the minimum recommended dietary allowance (RDA) for adults (0.8 g protein·kg^{-1} body weight day^{-1})[29] were instructed to increase their daily protein intake to this level by consuming additional protein from any source throughout the day. The increase in protein intake occurred during the 1-week wash-out period prior to the intervention.

Safety and Compliance

The safety of the resistance training program was assessed at every exercise session and testing time point through the identification and grading of adverse events using the common terminology criteria for adverse events (CTCAE v4.3). Muscle biopsy tolerability was assessed at a follow-up visit 48 to 96 hours post-biopsy and graded using CTCAE (v4.3). In addition, testosterone and prostate-specific antigen, assessed at baseline, intervention midpoint and post-intervention, were used as circulating safety measures of disease activity.

Resistance training attendance was calculated as the number of sessions completed compared to the total number of sessions. Resistance training adherence was calculated as the percentage of exercises performed at the prescribed modality, intensity, and volume as specified in the periodization model compared to the total number of exercises. Home-based flexibility program adherence was assessed by the physical activity log.

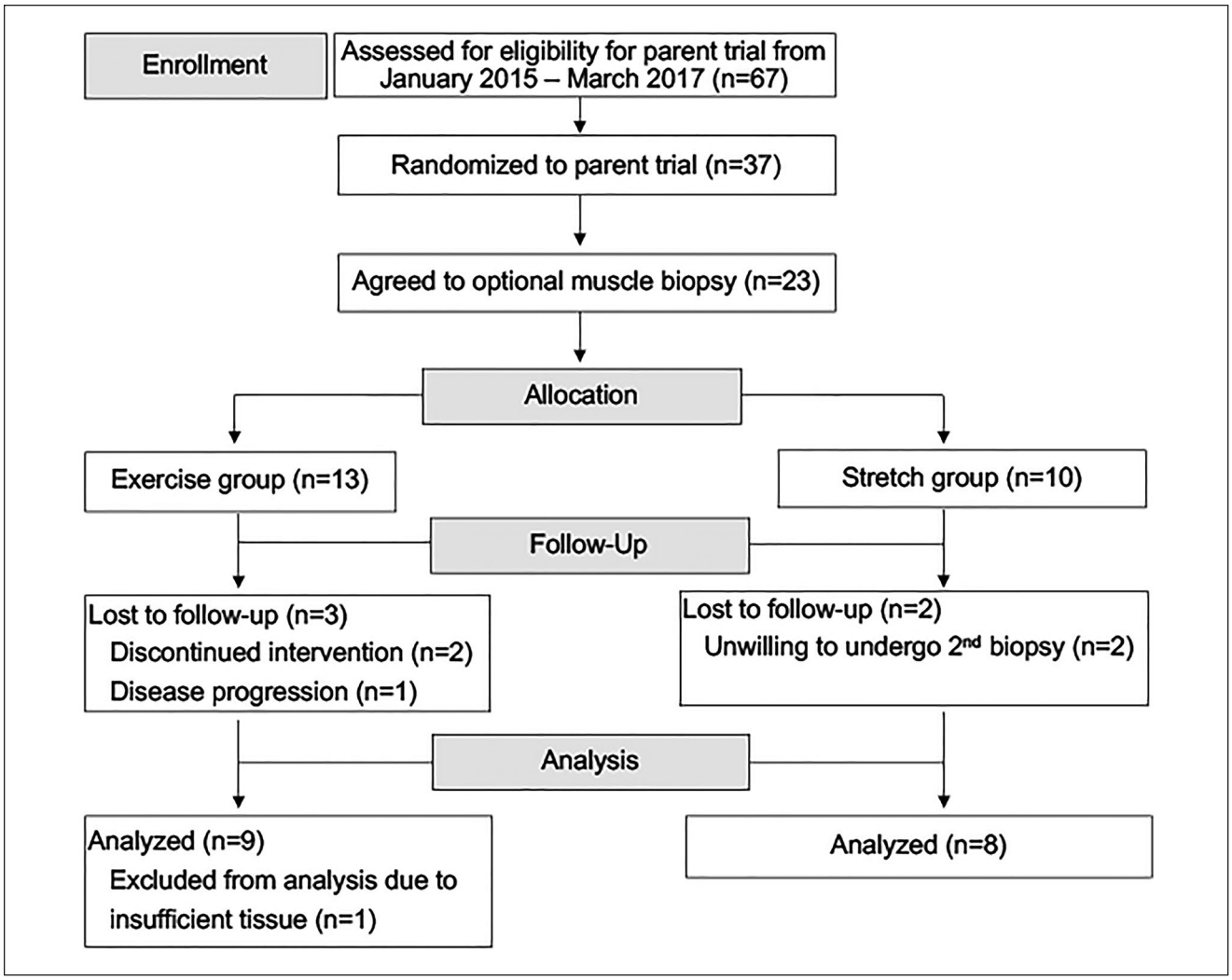

Figure 1. CONSORT diagram.

Statistical Analyses

A sample size estimation was not performed specifically for this study as it was an exploratory analysis of a larger pilot trial. However, a sample size of 32 participants was estimated in the parent trial to test the primary outcome hypothesis and inform effect sizes for a future RCT.[16] Only participants with baseline and post-intervention biopsies were included in the analysis. Because data for most variables were not normally distributed, outcomes are presented as Median (IQR) and nonparametric analyses were performed. Comparisons of baseline characteristics and pre-to-post changes between groups were made using Wilcoxon rank-sum tests for continuous outcomes and Fisher's exact test for categorical outcomes. For within-group baseline to post-intervention comparisons, the difference (post-pre) was computed on individual scores with Wilcoxon signed-rank tests used to assess significance. Changes in mRNA expression were correlated with changes in lean mass and strength outcomes in the EXE group using Spearman correlation, R_s. Effect size, r, was calculated from Wilcoxon rank-sum or signed-rank test statistics,[30] where 0.1 to 0.3 is considered a small effect, 0.3 to 0.5 a medium effect, and $\geq$0.5 a large effect.[31] Analyses were performed in R Studio (version 1.2.1335, Boston, MA, USA).

Results

Twenty-three of the 37 participants enrolled in the study consented to the optional muscle biopsy (Figure 1). No significant differences in baseline characteristics were observed between participants in the parent study and participants who volunteered for the muscle biopsy. In total, post-intervention muscle samples were not obtained from 3 EXE participants because of intervention discontinuation (n = 2) or disease progression (n = 1), and 2

Table 2. Baseline Characteristics.

	Exercise (n = 9)	Stretch (n = 8)	*P*	*r*
Age (yr)	64.0 [62.0, 72.0]	65.5 [60.5, 72.5]	.99	0.00
Height (cm)	170.2 [167.6, 178.6]	171.8 [165.4, 176.5]	.99	0.19
Weight (kg)	82.6 [73.0, 98.7]	76.1 [69.4, 84.2]	.42	0.00
BMI ($kg \cdot m^{-2}$)	27.5 [25.1, 34.2]	23.8 [22.8, 29.4]	.25	0.29
ADT duration (mo)	11.0 [3.0, 12.0]	11.5 [7.0, 25.5]	.29	0.26
Gleason score	8.0 [6.5, 8.5]	7.0 [7.0, 8.5]	.99	0.00
Time since diagnosis (yr)	2.9 [1.1, 5.2]	4.9 [1.1, 12.2]	.99	0.00
Total testosterone ($nmol \cdot l^{-1}$)	1.0 [0.1, 3.4]	1.3 [0.2, 2.1]	.69	0.10
PSA ($ng \cdot mL^{-1}$)	0.02 [0.01, 4.8]	0.09 [0.0, 1.2]	.96	0.01
Anti-androgen, n (%)	4 (44.4%)	4 (50.0%)	.99	
Previous radiation, n (%)	4 (44.4%)	5 (62.5%)	.64	
Previous surgery, n (%)	6 (77.8%)	7 (75.0%)	.99	
Previous chemotherapy, n (%)	1 (11.1%)	1 (12.5%)	.99	
Metastases, n (%)	6 (66.7%)	4 (50.0%)	.64	
Bone, n (%)	4 (44.4%)	4 (50.0%)	.81	
Protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	0.79 [0.75, 0.95]	0.87 [0.65, 1.12]	.81	0.06
Caloric intake ($kcal \cdot day^{-1}$)	1885 [1539, 2067]	1565 [1519, 1834]	.37	0.24
Total PA ($MET\text{-}min \cdot wk^{-1}$)[a]	1620 [1072, 2911]	3093 [1487, 4061]	.46	0.18
Moderate PA ($MET\text{-}min \cdot wk^{-1}$)	690 [310, 1560]	1920 [465, 2205]	.41	0.20
Vigorous PA ($MET\text{-}min \cdot wk^{-1}$)	100 [0, 562]	0 [0, 390]	.75	0.10

Data presented as median [interquartile range] or number of participants (% of participants). *P*, significance level; *r*, effect size.
Abbreviations: ADT, androgen deprivation therapy; BMI, body mass index; PA, physical activity; PSA, prostate-specific antigen.
[a]Includes walking, moderate, and vigorous activity.

STRETCH participants who declined to undergo the second biopsy. In addition, 1 EXE sample was excluded from the final analysis because of insufficient tissue quantity. No adverse events were reported due to the biopsy procedure.

In our study sample, 47% were non-Hispanic white, 59% had metastatic disease, 43% were classified as sarcopenic, and 41% met the criteria for metabolic syndrome at baseline. No significant differences were found between groups for any baseline characteristics ($P > .25$, $r < .29$; Table 2). Protein intake normalized to body weight (kg) was not significantly different between EXE (1.15 [0.91, 1.38] $g\,kg^{-1}\,day^{-1}$) and STRETCH (1.10 [0.84, 1.39] $g\,kg^{-1}\,day^{-1}$) over 12 weeks ($P = .81$, $r = .06$). Sensitivity analyses in only those participants who received the protein supplement (4 in EXE, 4 in STRETCH) were consistent with these findings, as there were no significant differences in protein intake between groups at baseline ($P = .69$, $r = .14$) or throughout the 12-week intervention ($P = .11$, $r = .50$), supporting the collapse of the 4-arm design into a 2-group analysis.

No adverse events were reported due to the exercise or stretching program. Exercise program compliance was 93.8%, with an average completion of 33 of 36 exercise sessions. Compliance to the home-based stretching program was 79.0%, with an average of 28 out of 36 sessions completed.

mRNA Expression Levels

Compared to STRETCH, the EXE group exhibited significantly greater fold change post-intervention for MuRF-1 expression ($P = .005$, Figure 2). However, no significant differences were observed between EXE and STRETCH post-intervention in fold change ($P > .05$, Figure 2) for myogenin ($r = .21$), PGC-1α4 ($r = .22$), IGF-1 ($r = .13$), or myostatin ($r = .37$).

Body Composition

Compared to STRETCH, EXE participants significantly increased appendicular skeletal mass after the 12-week intervention ($P < .05$, Table 3). In addition, the EXE group increased lean mass and appendicular skeletal mass from baseline to post-intervention ($P < .05$). No significant changes in other body composition parameters were observed in the EXE or STRETCH groups.

Physical Function

EXE participants significantly increased lower extremity strength post-intervention compared to STRETCH ($P < .01$, Table 4). While no significant differences were observed between EXE and STRETCH post-intervention for physical function outcomes ($r < .3$), EXE participants significantly

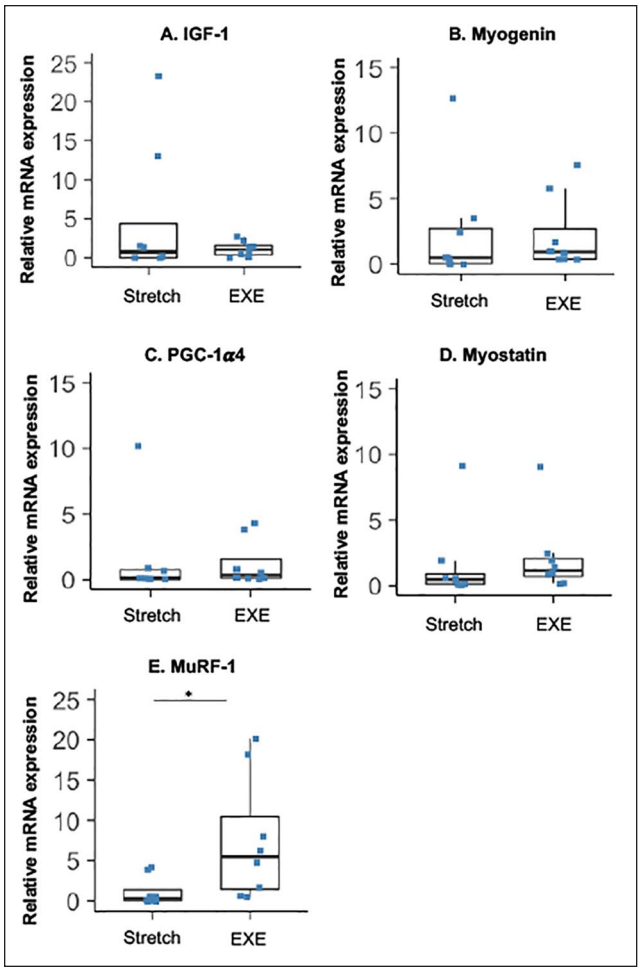

Figure 2. mRNA expression of (a) IGF-1, (b) myogenin, (c) PGC-1α4, (d) myostatin and (e) MuRF-1 presented as fold changes relative to baseline values and normalized to GAPDH. Data points represent individual change from baseline normalized to GAPDH. Box plots represent the median (dark horizontal line), interquartile range (box), and range (whiskers). Muscle biopsies were obtained at rest pre- and post- the 12-week intervention period. *Significantly different between stretch and exercise groups ($P < .05$), large effect size ($R = .67$).

improved Timed Up and Go ($P < .01$, $r = .96$) and stair climb ($P < .01$, $r = .89$) from baseline to post-intervention.

mRNA Expression Correlations

Changes in MuRF-1 expression were positively correlated with change in leg press ($R_s = .53$, $P = .036$), change in leg curl ($R_s = .63$, $P = .009$), and negatively correlated with change in stair climb time ($R_s = -.62$, $P = .013$). Change in lean mass was positively correlated with changes in IGF-1 expression ($R_s = .58$, $P = .015$). No other significant correlations were observed between mRNA expression fold changes and body composition or physical function outcomes (Table 5).

Discussion

To our knowledge, this is the first randomized controlled pilot study investigating the effects of a 12-week periodized resistance training program on mRNA expression of anabolic and catabolic regulators of skeletal muscle mass in PCS on ADT. Contrary to our hypothesis, resistance training was found to increase resting mRNA expression of the catabolic gene, MuRF-1, while no increases in resting levels of the anabolic genes IGF-1, myogenin and PGC-1α4 or decreases in the catabolic gene myostatin were observed. The change in MuRF-1 mRNA expression was correlated with changes in lower extremity muscular strength and physical function, while change in IGF-1 mRNA expression was correlated with change in lean mass.

Examination of skeletal muscle mRNA expression in PCS is limited, and we are not aware of other data investigating resting MuRF-1 mRNA expression following resistance training in PCS during ADT. Therefore, based on previous resistance exercise studies in healthy young males, we expected reductions in MuRF-1 mRNA expression post-training.[11] Contrary to our hypothesis, resting levels of MuRF-1 increased following 12 weeks of periodized hypertrophy resistance exercise. Though our findings diverge from studies employing traditional resistance exercise protocols with long rest periods (>2 minutes) between sets where resting levels of MuRF-1 mRNA expression were unchanged,[11,32,33] our results are similar to those using high-intensity protocols with limited rest periods (≤1 minute).[32-34] In healthy young males, increases in resting MuRF-1 mRNA expression have been reported following 8 weeks of resistance training with minimal rest periods (<1 minute),[34] 7 weeks of multimodal resistance and aerobic exercise,[32] and 10 weeks of high-intensity interval cycle training.[33] These similar patterns of MuRF-1 mRNA expression suggest the role of metabolic stress in modulating molecular responses. Exercise entailing high metabolic stress, such as limited rest, high-intensity, or multimodal protocols, may act as a driver of similar intracellular signaling and gene targets.[33] In the present study, multi-joint exercises were performed with a moderate number of maximal repetitions (6-12) for multiple sets and limited rest periods consistent with a hypertrophy training protocol.[35] As hypertrophy training regimens are purported to produce significant metabolic stress,[35] the elevated resting levels of MuRF-1 observed post-training in the present study may be due to the limited rest periods and time under tension consistent with a hypertrophy-oriented program.

We found that lower extremity strength was greatly enhanced by 12 weeks of resistance training, and observed a small, but significant difference in appendicular skeletal mass between groups post-intervention. Increases in strength directly correlated with increases in MuRF-1 mRNA expression, but no relationship was found between

Table 3. Changes in Body Composition Over 12 Weeks.

	Baseline	12 wk	*P* value[a]	Effect size	Change[b]	*P* value[c]	Effect size
Weight (kg)							
Exercise	82.6 [73.0, 98.7]	85.5 [71.1, 96.0]	0.23	0.40	1.3 [−0.1, 2.3]		
Stretch	76.1 [69.4, 84.2]	76.5 [69.3, 87.2]	0.99	0.00	−0.3 [−1.2, 2.6]	0.63	0.12
Lean mass (kg)							
Exercise	52.5 [47.3, 53.8]	53.0 [47.8, 55.7]	0.02	0.78	1.0 [0.5, 2.3]		
Stretch	48.9 [46.9, 50.0]	48.7 [45.7, 51.9]	0.99	0.00	−0.6 [−1.0, 1.1]	0.11	0.38
Appendicular skeletal mass (kg)							
Exercise	23.7 [20.5, 26.9]	24.3 [21.3, 27.6]	0.01	0.84	0.7 [0.3, 1.3]		
Stretch	21.2 [20.9, 22.6]	21.6 [20.2, 23.3]	0.94	0.02	−0.3 [−0.6, 0.6]	0.046	0.48
Fat mass (kg)							
Exercise	30.2 [23.2, 39.3]	26.7 [21.8, 40.0]	0.91	0.04	0.4 [−1.4, 1.4]		
Stretch	23.4 [19.6, 32.4]	24.8 [20.6, 32.0]	0.84	0.07	−0.1 [−0.5, 1.5]	0.61	0.13
Body fat (%)							
Exercise	38.3 [34.2, 41.7]	33.5 [31.3, 41.8]	0.18	0.44	−0.7 [−2.8, 0.1]		
Stretch	32.6 [30.4, 39.8]	33.6 [31.2, 38.8]	0.64	0.17	0.0 [−0.7, 1.7]	0.15	0.35

Data presented as median [interquartile range].
[a]Significance level for within-group change from baseline to 12 weeks analyzed by Wilcoxon signed rank test.
[b]Within-group (12-week—baseline) difference.
[c]Significance level for between-group changes analyzed via Wilcoxon rank sum test.

Table 4. Changes in Muscular Strength and Physical Function Over 12 Weeks.

	Baseline	12 wk	*P* value[a]	Effect size	Change[b]	*P* value[c]	Effect size
Leg press (kg)							
Exercise	105.4 [80.3, 166.5]	198.2 [150.6, 264.1]	<0.01	0.89	81.6 [66.0, 108.8]		
Stretch	83.7 [37.1, 146.7]	69.0 [39.4, 146.1]	0.27	0.39	−2.4 [−9.5, 1.1]	<0.01	0.92
Leg extension (kg)							
Exercise	93.0 [72.6, 105.5]	138.3 [115.7, 152.1]	<0.01	0.89	43.0 [35.2, 56.7]		
Stretch	61.2 [49.9, 76.0]	70.2 [60.0, 81.6]	0.27	0.39	9.0 [3.4, 12.4]	<0.01	0.80
Leg curl (kg)							
Exercise	61.2 [45.2, 77.2]	85.1 [70.4, 102.0]	<0.01	0.89	17.2 [14.8, 24.9]		
Stretch	52.2 [44.2, 59.0]	49.9 [40.8, 54.5]	0.92	0.04	0.0 [−8.0, 5.7]	<0.01	0.80
400 m walk (s)							
Exercise	230.0 [207.7, 274.6]	213.8 [204.0, 286.9]	0.25	0.38	−19.6 [−32.5, −2.1]		
Stretch	278.4 [233.4, 333.4]	283.5 [219.3, 345.0]	0.55	0.21	0.9 [−22.6, 39.3]	0.33	0.24
Timed up and go (s)							
Exercise	5.0 [3.9, 6.9]	4.1 [3.6, 6.1]	<0.01	0.96	−0.8 [−1.3, −0.4]		
Stretch	6.0 [4.6, 7.9]	3.2 [4.5, 8.0]	0.46	0.26	−0.3 [−1.1, 0.2]	0.24	0.29
Stair climb (s)							
Exercise	1.9 [1.6, 3.0]	1.7 [1.5, 2.4]	<0.01	0.89	−0.3 [−0.5, −0.1]		
Stretch	2.3 [1.9, 3.3]	2.1 [1.7, 3.6]	0.80	0.01	0.0 [−0.1, 0.7]	0.07	0.44

Data presented as median [interquartile range]. Strength values presented as 10-repetition maximum (RM) values.
[a]Significance level for within-group change from baseline to 12 weeks analyzed by Wilcoxon signed rank test.
[b]Within-group (12-week—baseline) difference.
[c]Significance level for between-group changes analyzed via Wilcoxon rank sum test.

appendicular skeletal mass and MuRF-1 mRNA expression. One explanation for these findings may be that we missed the window of anabolic gene expression and only captured the elevation in MuRF-1 expression. While elevations in proteolytic signaling such as that of ubiquitin proteasome pathway ligases like MuRF-1 are considered to be part of normal skeletal muscle remodeling during chronic exercise,[33,34,36] this degradation is countered by elevated rates of muscle protein synthesis, which, in long-term resistance training, is purported to lead to muscle protein accretion.[8] Activation of the anabolic remodeling process could have been occurring in parallel with the increases in strength due

Table 5. Correlations Between Changes in mRNA Expression and Changes in Skeletal Muscle Mass, Strength and Physical Function.

	IGF-1	PGC-1α4	Myogenin	MuRF-1	Myostatin
Lean mass (kg)	0.58 (0.015)*	0.12 (0.646)	0.37 (0.141)	0.25 (0.338)	0.26 (0.319)
ASM (kg)	0.15 (0.567)	−0.23 (0.379)	0.35 (0.171)	0.29 (0.256)	0.03 (0.918)
Fat mass (kg)	0.00 (0.985)	−0.20 (0.451)	0.08 (0.751)	−0.33 (0.195)	−0.17 (0.510)
Leg press (kg)	0.16 (0.557)	−0.01 (0.974)	0.09 (0.536)	0.53 (0.036)*	0.22 (0.582)
Leg extension (kg)	0.08 (0.782)	−0.02 (0.948)	−0.10 (0.725)	0.48 (0.057)	0.12 (0.660)
Leg curl (kg)	−0.04 (0.892)	0.01 (0.974)	0.10 (0.725)	0.63 (0.009)*	0.26 (0.336)
Timed up and go (s)	−0.10 (0.701)	−0.17 (0.523)	0.13 (0.619)	−0.32 (0.208)	−0.14 (0.593)
Stair climb (s)	−0.12 (0.680)	0.01 (0.960)	0.21 (0.455)	−0.62 (0.013)*	−0.15 (0.602)
400 m walk (s)	0.19 (0.478)	0.03 (0.905)	0.24 (0.362)	−0.28 (0.289)	0.05 (0.863)

Data presented as Spearman correlation R_s (significance level P).
Abbreviation: ASM, appendicular skeletal mass.
*$P < .05$, large effect size.

to neural adaptations, and if we had measured protein content, we might have observed this anabolic program. However, because only mRNA expression was measured, we captured changes in MuRF-1 levels, although strength improvements would not be attributable to elevations in MuRF-1 mRNA expression.

Missing the window of mRNA expression might explain why we did not observe the increases in resting levels of IGF-1 and myogenin or decreases in myostatin typically demonstrated in healthy older men or testosterone-suppressed younger men after a resistance training program.[9,10,14,37] Yet, other factors, such as timing of biopsies or testosterone status, might have played a role as well. In a previous investigation in young males on ADT, resting mRNA expression of IGF-1 and myogenin were found to increase and myostatin was found to decrease following 8 weeks of resistance training.[37] Biopsies were taken 24 hours after the last training session, which is contrast to the present study, where biopsies were performed 72 to 96 hours after the last training session. In the present study, this was done to avoid residual effects of training, as myogenin mRNA expression has been shown to be acutely elevated after a resistance exercise bout for up to 24 hours.[13] Testosterone status might also have been a factor in the divergent results. Resting mRNA expression of myogenin was increased in eugonadal older males after 21 weeks of resistance training.[14] However, eugonadal older men exhibit mean total testosterone levels of ~16 nmol L^{-1},[38] which is an order of magnitude greater than the PCS (~1 nmol L^{-1}) in the present study. As testosterone administration has been shown to increase IGF-1 mRNA expression in rat muscle,[39,40] it is possible that eugonadal testosterone levels potentiate the response of skeletal muscle IGF-1 mRNA expression to the anabolic stimulus of resistance exercise.[14]

The lack of change that we observed in PGC-1α4 mRNA expression parallels findings by Lundberg et al in eugonadal young men (~25 years) who performed 5 weeks of resistance training or combined resistance and aerobic training.[41] The unaltered resting levels despite increases in muscle cross-sectional area led the authors to conclude that resting PGC-1α4 expression was unlikely to contribute to hypertrophy. These findings are in contrast to Ruas et al, who noted elevated resting expression of PGC-1α4 following 8 weeks of resistance training in human samples collected 72 to 96 hours after the last training session. Marked increases in muscle mass and resistance to the muscle-wasting effects of cancer cachexia were also observed in transgenic PGC-1α4 mice, suggesting a role of PGC-1α4 in mediating an anabolic program of hypertrophy.[15] Although we did not observe significant increases in resting PGC-1α4 mRNA expression in PCS on ADT, without protein analysis we cannot exclude the involvement of PGC-1α4 in hypertrophic mechanisms.

We also observed no significant difference in myostatin mRNA expression between exercise and non-exercise groups post-intervention, which agrees with findings from Nilsen et al,[7] the only other study to quantify myostatin response to resistance training in PCS on ADT. Myostatin is a key modulator of both protein synthesis and degradation pathways, with inhibition of myogenin and activation of MuRF-1 among its diverse activities.[8] Despite observing increases in muscle cross-sectional area and strength, Nilsen et al[7] found no changes in resting myostatin protein content after 16 weeks of resistance training. These findings differ from previous reports in eugonadal young and older men,[9,10] where resting myostatin mRNA expression was reduced following 12 to 21 weeks of resistance training. Our results are similar to the unchanged resting myostatin mRNA expression observed in young men on ADT following 8 weeks of resistance training,[37] suggesting a role of androgen suppression in limiting the response of myostatin to exercise.

This study has several limitations. Importantly, this study was a pre-specified exploratory analysis of the clinical trial and was therefore underpowered to determine

between-group differences in mRNA expression. Also, alterations in the mRNA expression of anabolic and catabolic regulators of muscle mass may depend on many variables, including physical activity levels, nutritional status, and age.[24,42] While we attempted to control for differences between groups through the reporting and statistical analyses of these variables, it is possible that alternative intervening factors may exist that were not accounted for. We were only able to obtain small samples (~23 mg) from the vastus lateralis muscle, which prevented us from conducting other analyses such as protein content, fiber typing, and histochemical staining. The lack of protein expression data, in particular, limits our ability to draw conclusions about the contribution of anabolic genes during the muscle remodeling process that might not have been detected from mRNA analyses. In addition, other hypertrophic and proteolytic signaling genes besides those measured in the present study may contribute to the changes observed in strength and lean mass, although IGF-1 and myostatin were the genes primarily expected to regulate muscle mass. Other limitations of this study include a non-sedentary control group that might have blunted the effects of the intervention and biopsy time points that limit the generalization of these results to studies with similar collection times. In addition, heterogeneities in exercise program design among the studies discussed may explain the differences in findings. Hence, to unequivocally compare mRNA expression across studies, exercise protocols should be reproduced in the same patient population.

In conclusion, 12 weeks of resistance training increased resting MuRF-1 mRNA expression in *vastus lateralis* samples from prostate cancer survivors on ADT, but did not influence resting mRNA expression of myogenin, IGF-1, PGC-1α4, or myostatin. This randomized pilot trial was the first to investigate signaling through the IGF-1/Akt/mTORC1 and ubiquitin-proteasome pathways in the skeletal muscle of men with prostate cancer on ADT. Future studies can improve on this investigation by characterizing protein content as well as mRNA expression of anabolic, myogenic, and proteolytic genes in skeletal muscle of patients with prostate cancer on ADT and comparing these results to age-matched eugonadal men. Such investigations may aid in a greater mechanistic understanding of skeletal muscle regulation in the absence of androgens and the effects of resistance exercise and skeletal muscle mass on cancer-related outcomes.

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Ethics Approval

The study was approved by the University of Southern California (USC) Institutional Review Board (HS-13-00315) and registered at www.clinicaltrials.gov (NCT01909440). All participants provided written informed consent.

Trial Registration

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