Hormone therapy attenuates exercise-induced skeletal muscle damage in postmenopausal women

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Submitted 16 April 2009; accepted in final form 29 June 2009

Hormone therapy (HT) is commonly used in postmenopausal women to treat symptoms of menopause. However, little is known of the effects of HT on skeletal muscle function. A protective effect of endogenous estrogen against exercise-induced skeletal muscle damage may exist, but the effects of HT on exercise-induced skeletal muscle damage following eccentric exercise have not been examined. Previous studies have indicated that estrogen protects skeletal muscle against damage caused by high-intensity resistance exercise (20, 40). In general, decreased levels of creatine kinase (CK) are present in women after eccentric and sustained exercise compared with men (41), indicating a potential protective role of estrogen against muscle damage. Furthermore, women have lower CK levels at rest (25) and after endurance exercise (34).

Minimal evidence exists to demonstrate a protective effect of HT against skeletal muscle damage. Estrogen therapy in postmenopausal women after intense aerobic exercise did not offer a protective effect against exercise-induced skeletal muscle damage (13). Alternatively, a study in young women reported that oral contraceptive use lowers CK levels after eccentric exercise (9). An explanation of potential mechanisms and more conclusive results will help to determine the effects of estrogen/HT on exercise-induced skeletal muscle damage.

High-intensity eccentric exercise is widely used as a method of promoting skeletal muscle damage and is marked by loss of muscular strength, muscle soreness, and elevated blood concentrations of muscle proteins such as CK (11). Activities that involve eccentric muscle actions appear to be particularly disruptive to muscle structure. Evidence of myofibrillar disruption, inability to buffer calcium and Z band streaming in sarcomere structure, occurs in both high-intensity eccentric and concentric muscle actions; however, greater disruptions occur during eccentric muscle action (11). Primary skeletal muscle damage promotes infiltration by inflammatory cells (30, 31) to produce an array of cytokines to regulate the inflammatory process, including IL-6, 8, IL-15, and TNF-α (2, 22, 24, 42). These muscle-derived cytokines, commonly referred to as myokines (30), can be directly assessed by sampling muscle tissue to ensure that skeletal muscle damage is present (46). A single bout of exercise promotes increased circulation of IL-6, IL-8, IL-15, and TNF-α (28–31). Serum CK and serum lactate dehydrogenase (LDH) are often analyzed to indirectly assess the degree of muscle damage. High CK and LDH levels are associated with exercise-induced skeletal muscle damage. Elevated levels of CK and LDH following resistance exercise are reported 3–7 days after the exercise bouts (4, 12), including eccentric resistance training. Therefore, by measuring CK and LDH levels we can indirectly assess the degree of skeletal muscle damage in postmenopausal women.

Examsing the effects of eccentric resistance exercise on markers of skeletal muscle damage including CK, LDH, and mRNA expression of IL-6, IL-8, IL-15, and TNF-α in postmenopausal women using HT is unique in this population. The purpose of this investigation was to determine whether HT attenuates markers of exercise-induced skeletal muscle damage after maximal eccentric exercise in postmenopausal women. We hypothesized that in postmenopausal women, after a single bout of maximal eccentric exercise, HT attenuates skeletal muscle damage as reflected by decreased markers of exercise-induced muscle damage and inflammation.

METHODS

Study participants. Fourteen healthy, untrained (no participation in consistent, structured resistance or cardiovascular training) postmeno-
The participants were instructed to record their dietary intake over the course of 2 weekdays and 1 weekend day. Participants discussed their completed food record with a registered dietician from the Bio nutrition Department to determine accuracy in portion size estimates and detail of food intake. Analysis of the dietary record was performed by the registered dietician with Nutritionist Pro (Nutrition Analysis Software version 1.3, Jones and Bartlett Publishers, Sudbury, MA).

Strength testing. Strength testing was preceded by 5 min of warm-up on a cycle ergometer. Strength testing was performed with the Cybex Norm dynamometer (Cybex International Ronkonkoma, NY). Peak concentric and eccentric torque of the knee extensors were performed with the dominant leg using the Cybex Norm dynamometer at 60°/s. The Cybex was calibrated immediately before the exercise bout. Participants were positioned on the Cybex by visually aligning the placement of the lateral femoral condyle with the axis of rotation for the Cybex. A strap was placed distally on the dominant leg at the level of the load cell. The load cell was positioned 3 cm proximal to the talocrural joint. During the maximal loading, a shoulder harness, a hip restraint, and a thigh strap (exercised leg) were used to limit excessive movement and secure the participant to the device. The settings were recorded to ensure exact placement for each exercise bout.

The tester determined the participants’ peak eccentric and concentric torque. This was determined to assess whether the participants were generating maximal effort during each set of the acute training bout. Participants received detailed instructions on the eccentric and concentric leg extension exercises and performed no more than five repetitions of each exercise to achieve peak torque. Strength testing was performed once because of recent indications that repeat strength testing may not be necessary to accurately assess maximal strength (39). Participants recorded and orally confirmed a modified Borg soreness scale (5) 2–3 days after the strength testing.

Acute eccentric resistance training bout. The participants completed one acute bout of single-leg knee extension of maximal eccentric isokinetic loading on the dominant leg with the Cybex Norm dynamometer (Cybex International) at 60°/s during visit 4. The Cybex was calibrated immediately before the exercise bout. Participants were positioned on the Cybex with the settings recorded from the strength testing visit. The exercise protocol consisted of 10 sets of 10 maximal eccentric repetitions, with the participants performing the eccentric component while the investigator performed the concentric component by moving the limb back to the starting position (15° degrees of knee flexion). Each repetition was separated by the time it took the testing investigator to manually return the lever arm back to 15° degrees of knee flexion (i.e., starting position). Each of the 10 sets was separated by 20 s. Biofeedback was provided on the computer monitor, and verbal encouragement was provided by the tester during each maximal contraction. The training bout occurred ~2 wk after strength testing and 1 wk after the baseline muscle biopsy to allow recovery from soreness. Participants recorded and orally confirmed a modified Borg soreness scale (5) 2–3 days after the training bout.

Blood draws and assays. Venous blood was collected from the antecubital vein by standard sterile procedures at the USC GCRC and was analyzed by the Los Angeles County-USC Clinical Laboratory for chemistries and blood counts. Two blood draws were performed. Blood draw 1 was performed during preentry visit 1, and blood draw 2 was performed during visit 5. Participants fasted for 6–8 h before each blood draw. CK and LDH were measured with immunoassays in the USC Core Laboratory with an automated immunoassay analyzer (Immulite 1000, Siemens Healthcare Diagnostics, Deerfield, IL).

Muscle biopsies. Percutaneous muscle biopsy samples (150–200 mg) were obtained from the vastus lateralis of the exercised leg 1 wk before the acute exercise bout (visit 3) and 4 h after exercise (visit 4). Four hours after exercise is an appropriate time point to assess changes in mRNA expression stimulated by exercise (22, 48). Biopsy specimens were collected under sterile conditions and local anesthesia (1% lidocaine) with a 5-mm Stille biopsy needle (Micrins Surgical, Inc., Chicago, IL).
Lake Forest, IL) from the midportion of the vastus lateralis muscle. The postexercise biopsy was performed at a distance of 2–4 cm proximal to the first site. Muscle tissue samples were immediately flash-frozen in liquid nitrogen and stored at −80°C until being processed for analysis.

**RNA extraction and cDNA synthesis.** Total RNA was isolated after homogenization (Kinematica Polytron PT1200C) of 30–40 mg of muscle tissue with a monophasic solution including guanidine isothiocyanate-containing lysis buffer and β-mercaptoethanol. The concentration and purity of the RNA were determined with a UV spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Waltham, MA) by measuring absorbance at 260 nm and 280 nm. Five hundred nanograms of total skeletal muscle RNA was reverse transcribed to synthesize cDNA with Taqman reverse transcription reagents according to the manufacturer’s instructions (Applied Biosystems, Branchburg, NJ). RNA was reverse transcribed into cDNA with the following temperature/time protocol: 25°C for 10 min, 48°C for 30 min, 95°C for 5 min, and 4°C infinite (Myrcycler, Bio-Rad, Hercules, CA).

**Oligonucleotide primers for PCR.** Oligonucleotide primers were used to amplify the mRNA expression of IL-6, IL-8, IL-15, and TNF-α. Primer sequences were designed with the Primer3 program (35). The primer sequences for the specific target mRNAs and melt curve temperatures are shown in Table 1. Melt curves were determined for each primer set to ensure amplification of pure PCR products. GAPDH was used as an internal control for detecting mRNA quantification.

**Quantitative real-time PCR.** A qRT-PCR method was applied to determine relative expression levels of mRNAs for IL-6, IL-8, IL-15, and TNF-α. A total of 10 ng of cDNA was added to each of the 20-μl PCR reactions for IL-6, IL-8, IL-15, TNF-α, and GAPDH. Specifically, each PCR reaction contained the following mixture: 10 μl of 2.5× iQSYBgreen supermix (Bio-Rad), 7 μl of RNAse-free water, 10 ng of cDNA, and 10 pmol of each primer (forward and reverse) of interest. All samples were run in quadruplicate. Each PCR reaction was amplified with the Bio-Rad iCycler iQ thermal cycler (Bio-Rad). Thermal cycling conditions were specified by the manufacturer.

**mRNA quantification.** All data were determined by normalizing the cDNA measured in eight replicates (4 replicates repeated) for each participant sample to GAPDH (internal control) and then averaging the data to account for the change in mRNA expression as a result of the exercise stimulus. The data were then normalized to biopsy 1 (preexercise) for each participant to determine the fold change of mRNA expression after exercising. All participant samples were then averaged to determine the average mRNA expression before and after exercise for each group.

To test PCR efficiency, serial dilutions (1, 0.5, 0.250, 0.125, 0.062, 0.031) of cDNA for GAPDH and each gene of interest (GOI) were amplified by RT-PCR using gene-specific primers. Efficiency (efficiency = 10^[(1/slope)−1]) was calculated with the slope of the standard curve for each respective gene (17). The PCR efficiencies are 99% for GAPDH; 97% for IL-6, IL-8, and TNF-α; and 98% for IL-15. mRNA expression was evaluated by a relative quantification method (48). Briefly, this method is based on the fact that the difference in threshold cycles (ΔCt) between the GOI and the internal control genes (ICG) is proportional to the relative expression level of the GOI. The data were analyzed by using the 2^−ΔCt method (36) to compare relative mRNA expression (arbitrary units) between preexercise and postexercise biopsies, referred to as the fold change in mRNA expression.

**Statistical methods.** Statistical analyses were performed with SPSS version 16.0 (SPSS, Chicago, IL). Descriptive statistics and Pearson correlation coefficients were used to analyze the control and HT groups. Independent t-tests were performed to test group differences (control vs. HT). A probability level of P ≤ 0.05 was used to determine statistical significance. Group differences in mRNA expression were determined by repeated-measures analysis of covariance (ANCOVA) with weight, body mass index (BMI), BMD, bone mineral content (BMC), percent fat, fat mass, lean mass, protein intake, and carbohydrate intake as covariates. A priori analysis determined that a sample size of six participants in each group would have 80% power to detect a difference in means for MyoD mRNA expression of 2.0, assuming that the common standard deviation is 1.0 with a two-group t-test, with an effect size of 0.5 using a 0.05 two-sided significance level (32). We chose to determine our power analysis on the basis of changes in mRNA expression of MyoD based on MyoD mRNA expression previously examined in older women (32).

**RESULTS**

Baseline characteristics of the study participants are displayed in Table 2. The study results indicated that weight and BMI were significantly greater in the HT group compared with the control group (P < 0.05). There were no statistically significant differences between groups with dietary intake and muscle strength. One-repetition maximum (1-RM) strength testing values and peak torque generated during the acute training bout were not significantly different between the groups. There were no significant group differences in perceived muscle soreness. Average ratings of perceived muscle soreness for both groups were 5 (strong perceived soreness) out of 10 for the 1-RM testing and 8 (very strong perceived soreness) out of 10 for the acute exercise bout.

Although there were group differences in weight and lean mass, our results from ANCOVA indicated that participant characteristics were not significant covariates. Therefore, the data presented reflect results from independent t-tests for all outcome variables: CK, LDH, IL-6, IL-8, IL-15, and TNF-α.

### Table 1. Primer sequences used for qRT-PCR

<table>
<thead>
<tr>
<th>PCR Primer Sequence 5′→3′</th>
<th>Melt Curve, °C</th>
<th>Average Ct</th>
</tr>
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<tbody>
<tr>
<td><strong>IL-6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: CTA TGA ACT CCT TCT CGA CAA GGC CTT</td>
<td>82</td>
<td>21.3</td>
</tr>
<tr>
<td>R: GGG GGC GCT ACA TCT TGG GAA TCT T</td>
<td>82</td>
<td>20.5</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: GCT CTC TGT GAA GGT GCA GTT TGG CCA A</td>
<td>79</td>
<td>20.9</td>
</tr>
<tr>
<td>R: GGC GCA GTG TGG TCC ACT CTC AAT</td>
<td>85</td>
<td>19.6</td>
</tr>
<tr>
<td><strong>IL-15</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: CCG TGG CTT TGA GTA ATG AGA ATT TGG AA</td>
<td>79</td>
<td>18.3</td>
</tr>
<tr>
<td>R: CCT GCA CTG AAA CAG CCC AAA ATG AA</td>
<td>79</td>
<td>18.3</td>
</tr>
<tr>
<td><strong>TNF-α</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: CCC AGG CAG TCA GAT CAT CTT CTC GAA</td>
<td>79</td>
<td>18.3</td>
</tr>
<tr>
<td>R: CTT GGT ATC TCT CAG CTC CAC GGC ATT</td>
<td>79</td>
<td>18.3</td>
</tr>
<tr>
<td><strong>GAPDH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: AGC CAC ATC GCT CAG ACA</td>
<td>79</td>
<td>18.3</td>
</tr>
<tr>
<td>R: GGC CAA TAG GAC CAA ATG C</td>
<td>79</td>
<td>18.3</td>
</tr>
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qRT-PCR, quantitative real-time PCR; F, forward; R, reverse; Ct, cycle threshold.
Table 2. Participant characteristics

<table>
<thead>
<tr>
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<th>Control (n = 6)</th>
<th>HT (n = 8)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>59.2±4.2</td>
<td>58.5±3.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>160.4±9.0</td>
<td>163.8±3.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.1±17.4</td>
<td>89.5±23.7*</td>
</tr>
<tr>
<td>BMI, kg/cm²</td>
<td>35.4±11.5</td>
<td>47.2±11.9</td>
</tr>
<tr>
<td>Percent fat</td>
<td>34.1±13.6</td>
<td>45.1±7.9</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>41.6±4.3</td>
<td>49.1±5.0*</td>
</tr>
<tr>
<td>Estrogen, pg/ml</td>
<td>54.7±5.3</td>
<td>392.6±81.0*</td>
</tr>
<tr>
<td>Eccentric peak torque, Nm</td>
<td>108.7±52.9</td>
<td>132.7±58.6</td>
</tr>
<tr>
<td>Concentric peak torque, Nm</td>
<td>75.3±19.9</td>
<td>84.4±28.7</td>
</tr>
<tr>
<td>Peak torque†, Nm</td>
<td>101.3±17.7</td>
<td>118.3±32.4</td>
</tr>
</tbody>
</table>

Data are means ± SD for n subjects. HT, hormone therapy; BMI, body mass index; LBM, lean body mass. *Significantly different from control group, P < 0.05; †measured during acute exercise.

Pre- and postexercise values for serum CK and LDH are displayed in Fig. 1. There were no significant differences in CK and LDH levels at rest between the two groups (P > 0.05). After exercise, CK and LDH significantly increased in the control group compared with baseline levels (P < 0.001). After exercise, CK and LDH were not significantly elevated in the HT group compared with baseline levels (P > 0.001). The study results show significant increases from baseline in mRNA expression of TNF-α, IL-6, IL-8, and IL-15 in both the control and HT groups after exercise (P < 0.001, Fig. 2). TNF-α, IL-6, IL-8, and IL-15 mRNA expression were significantly greater in the control group compared with the HT group (P < 0.01).

DISCUSSION

We measured changes in markers of exercise-induced skeletal muscle damage including serum CK and LDH and mRNA expression of IL-6, IL-8, IL-15, and TNF-α in skeletal muscle following a maximal bout of eccentric exercise in postmenopausal women to determine whether HT provides a protective effect on exercise-induced skeletal muscle damage. In support of our hypothesis, after a maximal bout of eccentric resistance exercise HT attenuated skeletal muscle damage as indicated by decreased levels of serum CK and LDH and mRNA expression of IL-6, IL-8, IL-15, and TNF-α compared with postmenopausal women not using HT. Our study is the first investigation to examine exercise-induced skeletal muscle damage in postmenopausal women using HT by directly assessing mRNA expression in skeletal muscle tissue.

A potential protective role of estrogen against muscle damage has been proposed (20) and supported by the findings in our study with decreased levels of serum CK and LDH and mRNA expression of IL-6, IL-8, IL-15, and TNF-α compared with postmenopausal women not using HT. This protective role of estrogen may be the result of an interaction of three processes: 1) high antioxidant capacity, 2) membrane stabilizing properties, and 3) gene regulation (20). The effects of synthetic estrogens or HT on skeletal muscle damage have received minimal attention. Dobridge and Hackney demonstrated that postmenopausal women taking HT had elevated CK levels similar to those not taking HT after eccentric exercise, indicating a lack of a protective effect of estrogen on muscle damage (13).

Our study is not in accordance with Dobridge and Hackney (13) because our findings demonstrated significantly elevated levels of serum CK and LDH in the control group, where extremely low levels of estrogen were present. The HT group did not have elevated levels of CK and LDH, indicating the presence of a potential protective effect of estrogen against muscle damage. Since the HT group had greater lean mass than the control group, it may be possible that the HT group was stronger and the exercise protocol was not sufficient to induce muscle damage. However, as shown by the 1-RM data and peak torque produced during the exercise bout, the two groups were similar in strength and reported the same degree of muscle soreness. A potential limitation to our study was the
discrepancy in body mass between groups, and future studies may need to consider matching the groups for body mass.

Dobridge and Hackney (13) investigated estrogen use in nine postmenopausal women who performed a 30-min eccentric bout of treadmill running with a negative 10% grade at 70% maximal heart rate while taking HT and again after a 45-day washout period. There are two concerns with this study design, including an inadequate washout period and the use of an exercise protocol that may not be sufficient to induce muscle damage. The protocol employed in our study, 100 maximal eccentric knee extensions, has been used previously by our laboratory to successfully promote muscle damage (14, 19), unlike the protocol used by Dobridge and Hackney (13). Our study provides the first evidence of estrogen’s protective effect on muscle damage in two different groups of postmenopausal women with and without HT use.

Additionally, we measured markers of skeletal muscle damage directly from muscle tissue by examining mRNA expression of TNF-α, IL-6, IL-8, and IL-15 to accurately assess the effects of HT on skeletal muscle tissue. Cytokines released from muscle including IL-6, IL-8, IL-15, and TNF-α, more recently termed myokines, appear in skeletal muscle before inflammatory cells arrive (30). IL-6 mRNA expression increases after muscle contraction irrespective of glycogen depletion (22, 29, 37), as further indicated by our study, specifically to a greater degree in postmenopausal women not using HT. IL-6 may play a metabolic role, influencing glycogen levels in muscle (15). Specifically, IL-6 may increase glucose uptake by increasing GLUT4 translocation (8), and this relationship may be enhanced after eccentric exercise in postmenopausal women not using HT. The role of IL-8 in skeletal muscle after exercise remains unclear, yet it may play an angiogenic role by interacting with the chemokine receptor CXCR2 in capillaries to increase capillarization (6). Several studies have shown that IL-8 immediately increases after resistance exercise (2, 22, 28), similar to our study. The effect of eccentric resistance exercise on IL-8 mRNA expression was enhanced in postmenopausal women not using HT, which may lead to greater angiogenic effects compared with postmenopausal women using HT.

IL-15 blunts proteolysis and apoptosis in skeletal muscle in animal and human models (7, 16). Limited data exist on IL-15 activity in response to exercise. Nieman et al. (27) failed to observe any change in IL-15 mRNA expression from skeletal muscle tissue immediately after resistance exercise (10). However, our study demonstrated increased mRNA expression of IL-15 4 h after exercise consisting of 10 sets of 10 maximal repetitions of eccentric knee extension contractions in both study groups. This supports our contention that we employed an appropriate protocol to induce skeletal muscle damage following an acute high-intensity resistance exercise bout. Nieman et al. (27) utilized a resistance exercise protocol of moderate intensity, compared with our protocol, that included 4 sets of 10 repetitions at 40–60% of the subject’s 1-RM. Our exercise protocol was extremely strenuous on the quadriceps muscle group; therefore, muscle biopsies of the vastus lateralis were appropriate to demonstrate change in mRNA expression. The exercise protocol by Nieman et al. (27) involved both upper and lower body exercises and may not have provided a great enough stimulus to the vastus lateralis, also the sampling site for their muscle biopsies. However, a potential limitation of our exercise protocol was the use of exclusively maximal eccentric actions that are not traditionally performed, unlike the protocol used by Nieman et al. (27).

TNF-α is associated with trauma to the muscle and leads to muscle catabolism in human models (33). TNF-α mRNA modestly elevates with resistance exercise (22, 27, 38) and plays a role in initiating the breakdown and removal of damaged muscle fragments (43). Strenuous physical activity, including eccentric contractions, induces an inflammatory response that elevates TNF-α in skeletal muscle tissue (22, 26, 47). The increased levels of TNF-α mRNA expression following exercise in the postmenopausal women not using HT provides direct evidence that skeletal muscle damage occurred in response to the eccentric exercise bout. The increased mRNA expression of TNF-α supports our additional myokine findings that HT attenuates exercise-induced skeletal muscle damage in postmenopausal women.

Our findings demonstrate that HT use in postmenopausal women attenuated exercise-induced skeletal muscle damage measured in both serum and mRNA expression of skeletal muscle tissue. However, this study further emphasizes the need for additional research to determine specific benefits and/or risks of decreased markers of skeletal muscle damage as a result of HT use. The attenuation of skeletal muscle damage may play an inhibitory role in the natural inflammatory and immune response involved in the repair process following damage to skeletal muscle. This would delay the repair process and increase the time for return to normal function. The attenuation of skeletal muscle damage with HT use affected myokines normally involved in the inflammatory process, as indicated by the decreased levels of mRNA expression of TNF-α, IL-6, IL-8, and IL-15. These myokines serve other important functions in skeletal muscle including glucose uptake, angiogenesis, and myogenesis, which may be negatively affected by attenuation from HT use. Conversely, HT use may protect skeletal muscle from degeneration to prevent sarcopenia, which may minimize strength loss, risk of falls, and incidence of fractures. Long-term intervention studies focusing on HT use and resistance exercise are necessary to examine extended benefits on muscle growth and degeneration.

In conclusion, we determined that a single bout of maximal eccentric resistance exercise induced skeletal muscle damage in postmenopausal women not using HT and to a lesser extent in women using HT, as indicated by elevated serum levels of CK and LDH and mRNA expression of TNF-α, IL-6, IL-8, and IL-15. Postmenopausal women using HT experienced decreased elevation of markers of skeletal muscle damage, emphasizing a potential protective effect of HT against exercise-induced muscle damage. Overall, our study presents unique findings that provide insight into the effects of eccentric resistance exercise and HT use on exercise-induced skeletal muscle damage in postmenopausal women.

REFERENCES

MUSCLE DAMAGE IN POSTMENOPAUSAL WOMEN


