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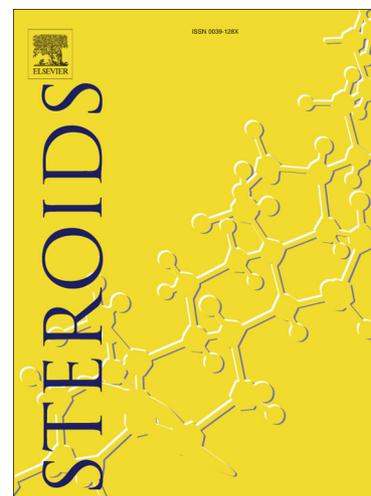
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Acute estradiol treatment reduces skeletal muscle protein breakdown markers in early- but not late-postmenopausal women

Running Title: Estradiol and muscle protein breakdown

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Abstract (Word count 246)

Objectives: Menopause and decline in estradiol (E_2) may contribute to sarcopenia (i.e., age-related decline in muscle mass and strength) in women. E_2 may directly impact skeletal muscle protein breakdown via estrogen receptor (ER) signaling, primarily $ER\alpha$. It is not yet known whether: 1) E_2 regulates pathways of skeletal muscle protein breakdown; 2) E_2 -mediated changes in protein breakdown markers are associated with $ER\alpha$ activation and insulin sensitivity; and 3) the effects of E_2 on protein breakdown markers differ by increasing time since menopause.

Study design: We studied 27 women who were ≤ 6 years past menopause (early postmenopausal, EPM; $n=13$) or ≥ 10 years past menopause (late postmenopausal, LPM; $n=14$). Fasted skeletal muscle samples were collected following 1 week of transdermal E_2 or placebo treatment in a randomized cross-over design.

Main outcome measures: We analyzed for cytosolic protein content of the: 1) structural proteins myosin heavy chain (MHC) and tropomyosin; and 2) protein regulatory markers: protein kinase B (Akt), muscle-specific ring finger protein1 (MuRF1), atrogin1, and forkhead box O3 (FOXO3) using Western blot.

Results: In response to acute E_2 , FOXO3 activation (dephosphorylation) and MuRF1 protein expression decreased in EPM but increased in LPM women ($p<0.05$). $ER\alpha$ activation was not associated with these protein breakdown markers, but FOXO3 activation tended to be inversely correlated ($r=-0.318$, $p=0.065$) to insulin sensitivity.

Conclusions: These preliminary studies suggest the effects of E_2 on skeletal muscle protein breakdown markers were dependent on time since menopause, which is consistent with our previous study on insulin sensitivity.

Key words: menopause, estradiol, skeletal muscle, protein breakdown, FOXO3

Abbreviations: estradiol (E_2), estrogen receptor (ER), hormone therapy (HT), early postmenopausal (EPM), late postmenopausal (LPM), placebo (PL), myosin heavy chain (MHC), protein kinase B (Akt), muscle-specific ring finger protein1 (MuRF1), forkhead box O3 (FOXO3), glucose disposal rate (GDR), oral glucose tolerance test (OGTT), Clinical and Translational Research Center (CTRC)

1. Introduction

Falls and hip fractures, and the ensuing loss of independent living are significant health issues in aging women. Older women experience greater rates of morbidity and physical disability than older men [1]. The age-related decline in muscle mass and strength (i.e., sarcopenia) contributes to this physical disability and increases health costs in this population [2]. Sarcopenia is not exclusively a condition of the very old, but instead occurs in ambulatory adults in middle age [3]. Although sarcopenia may begin in the third decade, changes in sex hormones and skeletal muscle protein metabolism become contributing factors in the fourth decade and beyond [3]. The decline in sex hormones during menopause in all women, as opposed to andropause which occurs in <10% of men during this time period [4], requires a closer examination of whether menopause contributes to sarcopenia in women. Lower lean mass in postmenopausal women compared to premenopausal women has been found in cross-sectional studies [5, 6] including the Study of Women's Health Across the Nation [5]. Furthermore, other studies reported that the decline in muscle strength in women begins around the time of menopause and occurs at a faster rate than in men [7]. It is important to elucidate the underlying mechanism(s) by which menopause contributes to the decline in skeletal muscle mass and identify the ovarian hormones associated with this muscular deterioration in women.

Increasing evidence suggests that estradiol (E_2) is an important factor in the menopause-related reduction in muscle mass. E_2 -based hormone therapy (HT) has been shown to preserve muscle mass and function in postmenopausal women [8, 9]. E_2 may directly impact myocytes via estrogen receptor (ER) signaling [10] and indirectly impact muscle mass and function through behavioral changes (i.e., free-living physical activity) [11]. Our recent work showed that acute E_2 treatment tended to increase the activation of $ER\alpha$ (i.e., translocation into the nucleus), with no change in $ER\beta$, in skeletal muscle tissues obtained from early (EPM, ≤ 6 years) and late (LPM, ≥ 10 years) postmenopausal women [12]. It is not known whether E_2 treatment alters skeletal muscle protein metabolism (i.e. protein breakdown), and whether this E_2 -mediated change in muscle protein metabolism is associated with $ER\alpha$ protein activation. Furthermore, our previous study [13] demonstrated that E_2 action on insulin-stimulated glucose disposal rate (GDR, i.e.

insulin sensitivity) was improved in EPM women but worsened in LPM women. These findings suggest that E₂ action on protein metabolism could also change with increasing time since menopause.

The PI3K and protein kinase B (Akt) pathway is a key regulator of muscle protein degradation [14], and can be activated (phosphorylated) by insulin and growth factors [15]. When activated, Akt inhibits protein degradation via multiple signaling branches including phosphorylation (deactivation) of cytosolic forkhead box O (FOXO) [16]. Conversely, deactivation of Akt results in dephosphorylation (activation) of FOXO and promotes its translocation to the myonucleus [17]. Nuclear FOXO activates the transcription of atrophic genes, including the atrogen-1 and muscle-specific ring finger protein1 (MuRF1) [18]. These atrogenes are components of the ubiquitin/proteasome machinery that degrades myofibrillar proteins, such as myosin heavy chain (MHC) and troponin [16]. A previous study [19] demonstrated lower expression of protein breakdown markers (i.e. FOXO3 and MuRF1) in early postmenopausal women using estrogen-based HT compared to non-HT users, suggesting an anti-catabolic role of E₂ in skeletal muscle. As a follow-up to our initial study [12] of skeletal muscle ER protein, the purpose of this study was to determine whether: 1) E₂ plays a critical role in signaling skeletal muscle protein breakdown in postmenopausal women; 2) E₂-mediated changes in markers of protein breakdown are associated with ER α activation; and 3) these changes differ by increasing time since menopause.

2. Experimental

2.1 Participants

We retrospectively analyzed skeletal muscle samples from a subset of postmenopausal women enrolled in our parent study [13] (13 EPM, ≤ 6 years of menopause; and 14 LPM, ≥ 10 years past menopause).

Participants were healthy, aged 45-70 years, non-obese (BMI $<30\text{kg/m}^2$), sedentary to moderately physically active, and with no history of using any formulation of estrogen-based HT. Detailed inclusion and exclusion criteria have been described previously [13]. The protocol was approved by the Colorado Multiple Institutional Review Board and participants provided written informed consent.

2.2 Experimental Design

This was a randomized, double-blinded, cross-over study wherein participants completed two treatment conditions: one week of transdermal placebo (PL) or E₂ (0.15mg/patch) separated by a six-week washout period. Oral glucose tolerance and body composition were measured before the first treatment. Specimens of vastus lateralis muscle were obtained prior to a hyperinsulinemic-euglycemic clamp on the last day of each treatment.

2.3 Glucose tolerance, insulin sensitivity, and circulating E₂

A 75g oral glucose tolerance test (OGTT) was administered in the morning following an overnight fast. Glucose tolerance was determined from the area under the curve for plasma glucose over two hours, using the trapezoidal method [13]. Whole body insulin sensitivity was measured in response to a hyperinsulinemic-euglycemic clamp as previously reported [13]. Plasma insulin and glucose samples from the OGTT and clamp tests were stored at -80°C and analyzed by the Colorado Clinical and Translational Research Center (CTRC) Laboratory. To demonstrate effectiveness of the transdermal treatment, serum levels of E₂ were measured using chemiluminescence (Beckman Coulter Life Sciences, Indianapolis, IN) by the CTRC Laboratory.

2.4 Body composition

Whole body fat-free mass and fat mass were measured by a dual x-ray absorptiometry (Hologic Discovery W, software version 11.2) as previously described [20]. Mid-thigh muscle areas were assessed by computed tomography as previously described [21]. Participants were asked to maintain body weight within ±2 kg throughout the study.

2.5 Three-day dietary lead-in

Participants consumed standardized meals (prepared by the CTRC metabolic kitchen) for the three days prior to each biopsy as previously reported [13]. Dietary composition was 50% carbohydrate, 34% fat, and 16% protein. The daily energy requirement to maintain body weight for each participant was determined using the Harris-Benedict equation [22].

2.6 Human muscle biopsy

Participants were instructed to avoid exercise for the 3 days prior to each muscle biopsy and to fast the night (~12 hr) before the biopsies. After sanitizing the skin in a sterile manner, 1% lidocaine was injected subcutaneously. A ~0.75 cm incision was made in the skin and fascia over the belly of the vastus lateralis, and a 5-mm Bergstrom side-cut biopsy needle was inserted into the muscle. Approximately 100 mg skeletal muscle specimens were obtained from one or more passes of the needle using suction. Specimens were cleaned of obvious adipose and blood, immediately flash frozen in liquid N₂, and later stored at -80°C.

2.7 Cellular protein fractionation

The cellular protein fractionation method was partially modified from previous studies [23]. Skeletal muscle (~30mg) was homogenized using a tissue homogenizer (Bullet blender, Next Advance, Averill, NY) in Buffer A containing 100mM NaCl, 20mM hepes, 50mM NaF, 1mM ethyleneglycol tetraacetic acid, 1mM ethylenediamine tetraacetic acid, 1mM dithiothreitol, 2mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride, 1mM sodium orthovanadate, 20ug/ml aprotinin, 20ug/ml leupeptin, 20ug/ml antipain, 5mM *p*-chloromercuri phenylsulfonate, 10mM iodoacetamide, and 6ul/ml phosphatase inhibitor cocktail 2&3; and centrifuged at 500g at 4°C for 5 min. Supernatant (cytosol fraction) was stored in -80°C.

2.8 Western blot

All targeted proteins were measured in the cytosolic protein fraction. Protein concentrations in the cytosolic fraction were assessed using the Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL). The protein homogenates (30 µg of protein) with laemmli buffer were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and transferred onto polyvinylidene difluoride membranes. Membranes were probed with primary antibodies (1:200-1,000 concentrations in 5% bovine serum albumin). Troponin antibody was obtained from Cell Signaling (#4002; Beverly, MA); Akt and pAkt from Santa Cruz Biotechnology (#8312, #33437, respectively; Santa Cruz, CA); MuRF1 and atrogin1 from ECM Biosciences (MP3401, #AP2041; Versailles, KY); MHC from R&D systems (mab4470,

Minneapolis, MN); FOXO3 from Abcam (#12162, Cambridge, MA); and pFOXO3 from Invitrogen (pa537578, Rockford, IL). Individual protein bands were quantified using a densitometer (Bio-Rad), and normalized to emerin (#5430, Cell Signaling, Beverly, MA) for the structural proteins, MHC and troponin [24], and to β -actin (#4967, Abcam, Cambridge, MA) for the other cytosolic proteins.

2.9 Statistical analysis

A two-group repeated measures design was used to test the main effects of group (EPM and LPM) and treatment (PL and E₂), and the group x treatment interactions. If a significant group x treatment interaction was found, LSD post-hoc tests were performed for pair-wise comparisons. Bivariate Pearson's correlations were used to test the association between protein expression and insulin sensitivity. Baseline group differences were assessed using *t* tests. All data were analyzed using IBM SPSS Statistics version 24.0. Data are reported as mean \pm SEM unless otherwise specified, and $P < 0.05$ was considered statistically significant.

3. Results

3.1 Participants

LPM women were 7 years older and 9 more years past menopause compared to EPM (Table 1). LPM women had less total body fat mass, fat-free mass, thigh muscle area, and body weight compared to EPM, but there was no group difference in percent body fat. All women had normal OGTT glucose and insulin concentrations with no significant group differences. Our parent study [13] showed a significant group x treatment interaction for GDR (i.e. whole body insulin sensitivity) such that E₂ treatment improved GDR in EPM but diminished GDR in LPM, compared to the PL. However, the present study with a smaller subset found no significant interaction ($P = 0.14$). There was no significant group difference in circulating E₂ level on the PL arm, but E₂ treatment significantly increased circulating E₂ level in both EPM and LPM ($P < 0.001$).

3.2 Muscle protein expression

We found no significant group or treatment effects on the expression of the structural proteins, MHC and tropomyosin (Figure 1). There were no significant group or treatment effects on Akt, pAkt, or the ratio of

pAkt/Akt protein expression (Figure 2A-C). Although there was no difference in FOXO3 expression between groups, we found a non-significant trend for pFOXO3 to increase following E₂ treatment in EPM and to decrease in LPM (group x treatment interaction, $P=0.160$; Figure 2D and E). The ratio of FOXO3/pFOXO3 (i.e. dephosphorylation) showed a significant group x treatment interaction ($P=0.027$; Figure 2F) such that E₂ decreased this ratio 20% in EPM but increased 68% in LPM. Similarly, there was a significant group x treatment interaction ($P=0.013$; Figure 3A) for MuRF1 protein whereby E₂ decreased MuRF1 31% in EPM but increased MuRF1 17% in LPM. We found no group differences or treatment effects on atrogin1 protein expression (Figure 3B). We found no group differences or treatment effects on our loading controls, emerin and β -actin (all, $p>0.05$).

3.3 Associations

The E₂-mediated change in FOXO3/pFOXO3 protein expression was not correlated with the E₂-mediated change in ER α activation (i.e. the ratio of nuclear/cytosolic ER α protein expression; $r=-0.037$, $P=0.880$; Figure 4A; ER α data were presented in our previous study [12]), but tended to be inversely correlated with the E₂-mediated change in GDR (i.e. whole body insulin sensitivity; $r=-0.318$, $P=0.065$; Figure 4B).

4. Discussion

The present study is the first to demonstrate that the effects of E₂ on markers of skeletal muscle protein breakdown were dependent on time since menopause, such that acute E₂ treatment decreased FOXO3 activation and MuRF1 protein expression in EPM but increased these catabolic markers in LPM. We hypothesized that the action of E₂ on signaling pathways for skeletal muscle protein breakdown would be mediated through ER α activation but this was not the case. Instead, our data suggest that the E₂-mediated change in FOXO3 activation was inversely correlated with the E₂-mediated change in insulin sensitivity. Lastly, the present study found no group differences or significant effects of E₂ treatment in the content of two skeletal muscle structural protein markers.

4.1 Estradiol treatment and skeletal muscle mass

Using an appendicular lean mass index (≤ 5.67 kg/m²) assessed by DXA, Newman et al. [25] showed that the prevalence of sarcopenia was approximately 20% in older postmenopausal women. The onset of

sarcopenia could be triggered by the menopause transition beginning in the fourth decade of age and progressing thereafter [3]. In some cross-sectional studies, menopause was associated with lower muscle mass [5, 6] and strength [7, 26]. It is becoming increasingly evident that E₂ may be an important factor in the development of sarcopenia in women. Studies of HT [8, 9] support the importance of E₂ for preserving lean body mass and skeletal muscle mass in postmenopausal women. Perhaps most compelling is the study [9] of postmenopausal, monozygotic twins in which one twin was treated with E₂-based HT for 7 years. The HT-treated twin had greater thigh muscle area, muscle power, and mobility relative to the twin not using HT. Because E₂ treatment for one week in the present study would have been too brief to elicit changes in skeletal muscle or whole body lean mass, we investigated skeletal muscle structural proteins (i.e. MHC and troponin) that would be targets of FOXO and downstream catabolic mechanisms, but found no E₂-related effects. A longer duration of E₂ treatment may be necessary to detect measurable changes in muscle structural proteins and skeletal muscle mass.

4.2 Estradiol treatment and muscle protein breakdown

Dephosphorylated FOXO in cytosol activates the transcription of two muscle-specific E3 ligases, atrogin-1 and MuRF1 [18]. Both enzymes are highly expressed in animal and human models of acute muscle atrophy, and regulate the ubiquitination-degradation process of both structural (i.e. MHC and tropomyosin) and regulatory (i.e. MyoD) proteins [27, 28]. In young men, atrogin-1 mRNA expression increased more than 2-fold, MuRF-1 mRNA expression increased 1.5-fold, and total ubiquitinated proteins increased 4-fold in response to 20 days of bedrest that resulted in muscle atrophy [29]. In response to leg immobilization (i.e., knee casting) for 2 weeks, quadriceps lean mass significantly decreased by 4.7% and atrogin-1 protein expression significantly increased by 62% compared to baseline levels in young men [30]. These studies suggest that the protein breakdown markers, FOXO3, MuRF1, and atrogin1, play a critical role in regulating muscle mass.

One of the most interesting findings in the present study is that the effects of E₂ on markers of skeletal muscle protein breakdown (i.e. FOXO3 activation and MuRF1 content) were dependent on time since menopause, such that we found anti-catabolic effects of E₂ (i.e. suppressing protein breakdown) in

women who were ≤ 6 years postmenopause but an adverse effect in women with ≥ 10 years postmenopause. Our findings in EPM women are in line with Dieli-Conwright et al. [19] who demonstrated lower expression of FOXO3 and MuRF1 in postmenopausal women aged 50-57 years (similarly aged to our EPM) using HT compared to non-users. It is more difficult to explain why E_2 would become pro-catabolic later in menopause. A pre-clinical study [31] using young (7-week-old) ovariectomized mice partially supports our findings of a catabolic effect of E_2 . A single intramuscular injection of E_2 valerate (0.1 mg/kg) caused an increase in ubiquitin-specific protease 19 (a critical enzyme of the ubiquitination-degradation process) protein expression, and a decrease in hindlimb muscle mass. However, this preclinical model of ovariectomy may not be a relevant comparison to the progressive changes in circulating sex hormones in the LPM women in the present study. To our knowledge, there have been no animal and human studies investigating the effect of E_2 treatment on markers of muscle metabolism with the initiation of $E_2 \geq$ several months post ovariectomy in mice or ≥ 10 years post menopause in women. Additional studies are necessary to delve into the mechanisms by which E_2 may become detrimental to skeletal muscle later in menopause.

4.3 Muscle protein breakdown, estrogen receptor, and insulin sensitivity

E_2 may directly impact skeletal muscle cells via $ER\alpha$ and/or $ER\beta$ signaling [10]. In rodents, selective knock out of skeletal muscle $ER\alpha$, but not $ER\beta$, led to impaired glucose and insulin metabolism [32, 33]. Our parent study, utilizing muscle specimens from the same participants as in the present study, showed that the ratio of $ER\alpha$ nuclear/cytosolic (a surrogate marker of ER activation; translocation into nucleus) tended to be increased ($p=0.07$) following E_2 treatment independent of time since menopause, and to be lower ($p=0.07$) with prolonged duration of estrogen deficiency (i.e. LPM) [12]. To our knowledge, no human study demonstrated whether time since menopause impacts skeletal muscle protein metabolism via $ER\alpha$. Contrary to our hypothesis, the present study found no significant correlation between protein breakdown (i.e. FOXO3 activation) and ER activation (Figure 4A), suggesting an alternative mechanism(s) linking E_2 treatment to catabolic pathways during menopause.

We previously demonstrated an insulin-stimulated improvement in systemic glucose disposal following short-term administration of E₂ or conjugated estrogens in early postmenopausal women [34, 35]. Our parent study [13] reported a negative effect of E₂ on whole body insulin sensitivity (i.e. GDR) with increasing time since menopause; a trend that parallels our findings in protein breakdown markers in the current study. Furthermore, we identified an inverse association, albeit non-significant ($P=0.065$), between whole body insulin sensitivity and FOXO3 activation, a marker of protein breakdown (Figure 4B). The relation between skeletal muscle protein metabolism and insulin sensitivity is still unclear. Protein turnover was not different in individuals with type 2 diabetes or insulin resistance compared to healthy individuals [36, 37]. Contrarily, other studies have found a significant increase in whole body proteolysis in type 2 diabetes and obese individuals [38, 39]. However, our studies of healthy postmenopausal women are not readily comparable to these previous studies. Future studies directly comparing E₂-mediated changes in insulin sensitivity and muscle protein metabolism between insulin-resistant women and controls in early- and late-menopause are needed to confirm this.

4.4 Potential limitations

There are limitations to the present study to be considered. The first important limitation was reliance on muscle samples obtained during the basal (fasting) state. Specimens obtained in the insulin-stimulated condition would have allowed more elaborate analyses of the protein synthesis pathways, including Akt signaling, in response to E₂ treatment. We found no group or treatment effects on phosphorylated (activated) Akt, possibly due to a lack of anabolic stimulus. Future studies are necessary to investigate whether E₂ impacts protein synthesis and signaling (e.g. mTOR, S6K1, and 4EBP1) when stimulated by insulin or essential amino acids. Second, this study is a secondary analysis of muscle specimens obtained as part of a larger clinical trial [13] powered to distinguish differences in insulin sensitivity in response to E₂ in early and late menopausal women. Because the parent study was not powered for protein expression, our present results should be interpreted cautiously and the investigation needs to be reproduced with a greater number of samples. Third, the nuclear protein marker, Lamin, was found in the cytosolic fraction of the muscle specimens, indicating nuclear contamination that may have occurred during freezing.

Because nuclear protein expression was measured in the cytosolic fractions, the results need to be considered representative of the whole myocyte rather than the cytosolic fraction. Lastly, the present study focused on the mechanistic role of E₂ in skeletal muscle protein breakdown of postmenopausal women. Future studies are necessary to investigate the role of E₂ in postmenopausal women with low versus normal muscle mass for body size, and when coupled with hypertrophic exercise interventions.

5. Conclusions

Our data suggest that E₂ administration may be anti-catabolic during early menopause but pro-catabolic later in menopause, and that these effects parallel changes in insulin sensitivity.

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Table 1. Participant Characteristics

	EPM	LPM
	n=13	n=14
Age (yr)	55 ± 3	62 ± 3 ^a
Time since menopause (yr)	3 ± 1	12 ± 2 ^a
Weight (kg)	69.4 ± 7.8	60.8 ± 6.1 ^a
BMI (kg/m ²)	26.0 ± 2.5	23.6 ± 3.0 ^a
Total fat mass (kg)	25.2 ± 5.4	20.9 ± 5.3 ^a
Fat-free mass (kg)	44.2 ± 4.4	39.8 ± 2.8 ^a
%Fat (kg/kg)	36.1 ± 4.7	34.1 ± 5.8
Right thigh muscle area (cm ²)	107.8 ± 11.1	97.1 ± 15.9 ^a
Left thigh muscle area (cm ²)	106.1 ± 12.5	94.9 ± 14.8 ^a
OGTT glucose AUC (*10 ⁴)	1.3 ± 0.2	1.4 ± 0.4
OGTT insulin AUC (*10 ³)	7.5 ± 3.9	6.4 ± 2.5
ΔGDR (mg/kgFFM/min)	0.2 ± 0.8	-0.5 ± 1.5 ^b
Serum E ₂ (pg/mL) - PL arm	15.2 ± 17.7	10.4 ± 6.6
- E ₂ arm	167.8 ± 96.1	148.1 ± 63.8

mean±SD; ^a p<0.05 group difference; ^b **p=0.14**; AUC, area under the curve; EPM, early (≤6yr) postmenopausal; LPM, late (≥10yr) postmenopausal; BMI, body mass index; ΔGDR, estradiol-mediated change in glucose disposal rate during clamp; OGTT, 2hr oral glucose tolerance test; FFM, fat-free mass; E₂, estradiol; PL, placebo.

Figure Legends

Figure 1. Skeletal muscle structural proteins. (A) myosin heavy chain (MHC) and (B) tropomyosin protein content. Values are means ± SE (early postmenopausal, EPM, ≤6yrs, n=13; and late postmenopausal, LPM, ≥10yrs, n=14). Group = main group effect (EPM vs LPM). Treatment = main treatment effect (estradiol, E₂ vs placebo, PL).

Figure 2. Skeletal muscle protein kinase B (Akt) and forkhead box O3 (FOXO3) protein. (A) Akt, (B) phosphorylated (p) Akt, (C) ratio of pAkt/Akt, (D) FOXO3, (E) pFOXO3, and (F) ratio of FOXO3/pFOXO3. Values are means ± SE (early postmenopausal, EPM, ≤6yrs, n=13; and late postmenopausal, LPM, ≥10yrs, n=14). Group = main group effect (EPM vs LPM). Treatment = main treatment effect (estradiol, E₂ vs placebo, PL). G x T = group x treatment interaction.

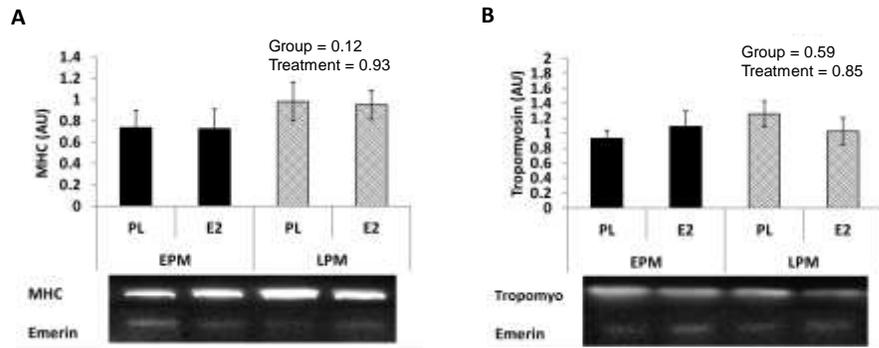
Figure 3. Skeletal muscle-specific E3 ligases. (A) muscle-specific ring finger protein1 (MuRF1) protein, and (B) atrogin1 protein content. Values are means ± SE (early postmenopausal, EPM, ≤6yrs, n=13; and

late postmenopausal, LPM, ≥ 10 yrs, $n=14$). Group = main group effect (EPM vs LPM). Treatment = main treatment effect (estradiol, E_2 vs placebo, PL). G x T = group x treatment interaction.

Figure 4. Associations. Estradiol (E_2)-mediated change in forkhead box O3 (FOXO3)/pFOXO3 protein ratio versus (A) E_2 -mediated change in estrogen receptor (ER) α nuclear/cytosolic protein ratio; and (B) E_2 -mediated change in whole body insulin sensitivity. Early postmenopausal (EPM, ≤ 6 yrs, $n=13$) and late postmenopausal (LPM, ≥ 10 yrs, $n=14$). *Denotes a trend for a significant correlation ($P=0.065$).

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Figure 1



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Figure 2

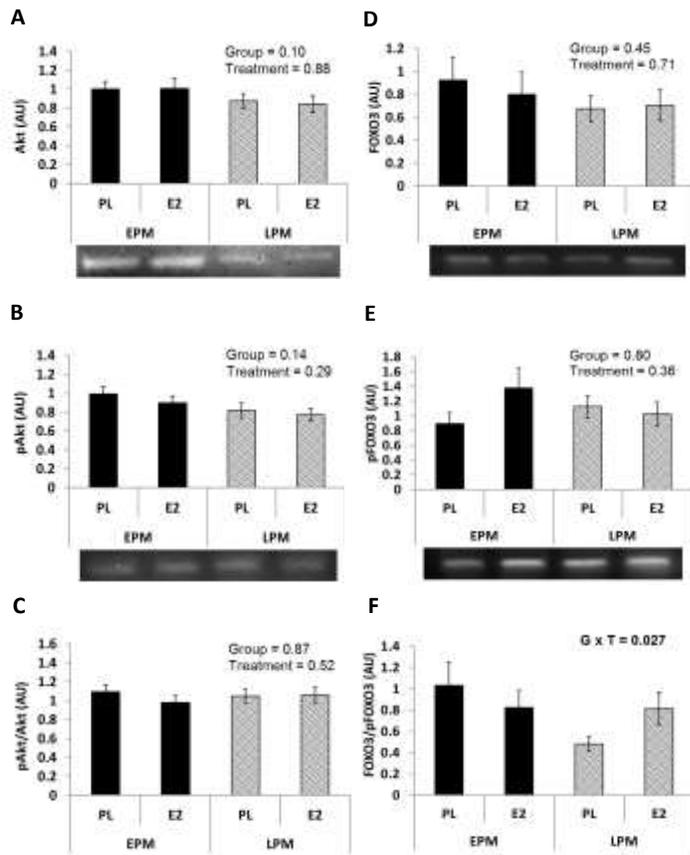
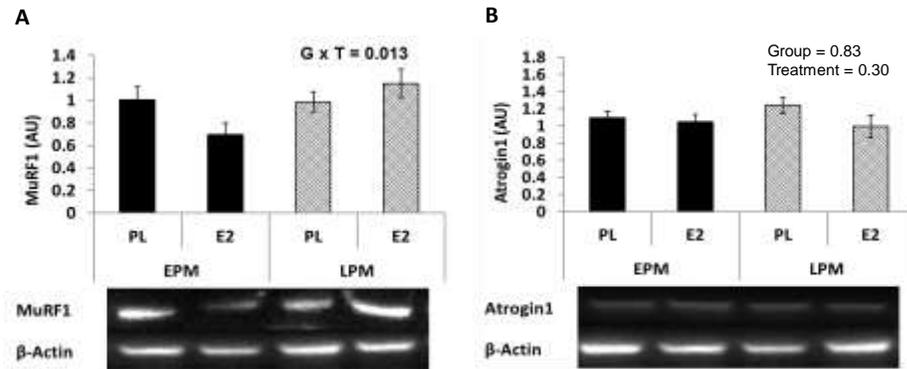


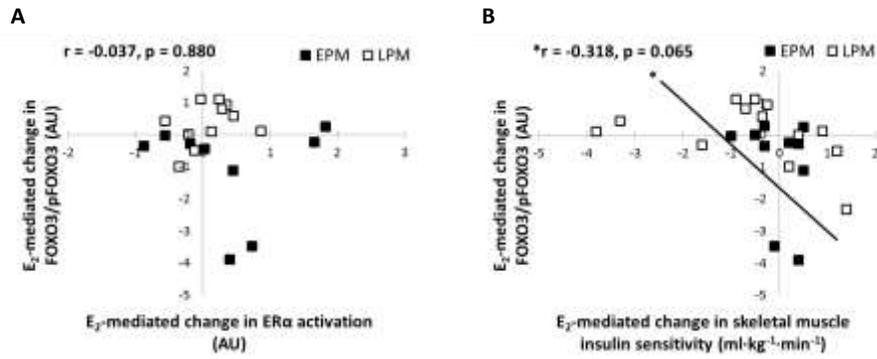
Figure 3



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Figure 4



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Highlights

- The acute E₂ effect on muscle protein breakdown is dependent on time since menopause
- The acute E₂ action for muscle protein breakdown is not related to estrogen receptor
- The E₂-mediated change in FOXO3 is inversely correlated with insulin sensitivity

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