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Author(s): Collins, Brittany C.; Laakkonen, Eija; Lowe, Dawn A.

Title: Aging of the musculoskeletal system : How the loss of estrogen impacts muscle strength

Year: 2019

Version: Accepted version (Final draft)

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Please cite the original version:

Collins, B. C., Laakkonen, E., & Lowe, D. A. (2019). Aging of the musculoskeletal system : How the loss of estrogen impacts muscle strength. *Bone*, 123, 137-144.

<https://doi.org/10.1016/j.bone.2019.03.033>

BONE Special Issue: Musculoskeletal Aging

Aging of the Musculoskeletal System: How the Loss of Estrogen Impacts Muscle Strength

Brittany C. Collins¹, Eija K. Laakkonen², and Dawn A. Lowe³

¹Department of Human Genetics, Medical School, University of Utah

²Gerontology Research Center and Faculty of Sport and Health Sciences, University of Jyväskylä

³Divisions of Rehabilitation Science and Physical Therapy, Department of Rehabilitation Medicine, Medical School, University of Minnesota

Abstract

Skeletal muscle weakness occurs with aging and in females this is compounded by the loss of estrogen with ovarian failure. Estrogen deficiency mediates decrements in muscle strength from both inadequate preservation of skeletal muscle mass and decrements in the quality of the remaining skeletal muscle. Processes and components of skeletal muscle that are affected by estrogens are beginning to be identified. This review focuses on mechanisms that contribute to the loss of muscle force generation when estrogen is low in females, and conversely the maintenance of strength by estrogen. Evidence is accumulating that estrogen deficiency induces apoptosis in skeletal muscle contributing to loss of mass and thus strength. Estrogen sensitive processes that affect quality, i.e., force generating capacity of muscle, include myosin phosphorylation and satellite cell function. Further detailing these mechanisms and identifying additional mechanisms that underlie estrogenic effects on skeletal muscle is important foundation for the design of therapeutic strategies to minimize skeletal muscle pathologies, such as sarcopenia and dynapenia.

Keywords: Aging; Estrogen; Skeletal muscle; Strength

Introduction

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Skeletal muscle weakness is an undesirable consequence of aging. Sarcopenia, the age-related loss of muscle mass and strength, has been identified as the crucial precursor to frailty, leading to disability, loss of independence [1], as well as hospitalization [2] for aged individuals. While decreased muscle mass contributes to sarcopenia, strength declines to a greater extent such that strength normalized for muscle size is reduced with age. Dynapenia is a relatively new term used to describe this age-associated loss of muscle strength that is independent of muscle atrophy [3]. The significance of dynapenia is exemplified by Visser and Schapp, who conclude that the loss of muscle strength consistently predicts falls, mortality and functional status, such as mobility in elderly individuals, while evidence for the relationship between low muscle mass and these outcomes is limited [4].

Skeletal muscle of females is dually affected by age due to the simultaneous loss of ovarian hormone production. In women, the production of estradiol and progesterone falls at menopause, occurring at 45-52 years of age [5, 6]. Declines in strength are accelerated at this age in women [7-13] and meta-analyses of nearly 10,000 post-menopausal women showed that those on hormone therapy (HT) had slightly greater muscle strength than those not on HT [14]. Such results point toward estrogen as being the key ovarian hormone affecting muscle strength in women. In this article, we will use the abbreviation HT to encompass both estrogen and combined estrogen-progesterone therapy in women as recommended by the 2017 North American Menopause Society position statement [15].

A systematic review and meta-analyses comparing force generation of muscle from ovarian hormone-deficient and –replete mice also indicate that muscle strength decrements occur when estrogen is deficient [14]. The most common method of inducing ovarian hormone deficiency in pre-clinical rodent research is ovariectomy, i.e., bilateral surgical removal of the ovaries, and this approach is often referred to as a model of menopause [16].

1 In rodents, estrous cycles become irregular and eventually cease with age. The age at which
2 this occurs is variable across strains of mice and rats. For example, cessation of estrous cycles
3 in C57BL6 mice was initially reported to occur between 13 and 16 months of age [17] and
4 was more recently measured to occur between 17 and 20 months of age [18]. The term
5 ovarian senescence is used to indicate when estrous cycles cease and this natural, aged-
6 induced loss of ovarian hormone production is more similar to menopause in women than
7 ovariectomy surgery (reviewed by [19]). Despite this more comparable physiology, the aged
8 female rodent model is infrequently used to analyze how the loss of estrogen detrimentally
9 affects muscle strength.
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21 The focus of this review will be on the putative mechanisms that contribute to the loss
22 of muscle force generation when estrogen is low in females, and conversely preserve strength
23 when estrogen is present (Figure 1). Studies on menopausal women and estrogen-deficient
24 and -replete rodents will be summarized as to how muscle strength is affected by estrogen,
25 and lack thereof. The specific estrogenic mechanisms considered in the review will be limited
26 to 1) regulation of skeletal muscle mass affected by estrogens and 2) processes in skeletal
27 muscle contraction, which if perturbed by estrogen deficiency will diminish the generation of
28 force.
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44 **1. Estrogen and skeletal muscle mass**

45 While the anabolic effects of androgens on skeletal muscle in males are recognized to
46 improve mass and thus strength (e.g., [20, 21]), by comparison there is much less known
47 about effects of estrogens on skeletal muscle mass in females [22]. Current evidence indicates
48 that estrogens have a role in maintaining muscle mass [23-25]. When estrogen is deficient, as
49 occurs with advanced age in females, muscle atrophy ensues and contributes to muscle
50 weakness. This section of the review will present evidence on cellular and molecular
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1 mechanisms by which estrogen may contribute to maintaining skeletal muscle mass and on
2 the contrary, mechanisms underlying how estrogen deficiency may cause atrophy. While age-
3 related weakness (dynapenia) can occur independent of atrophy, it is important to note that
4 loss of muscle mass may also contribute to loss of strength.
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9 ***Muscle protein turnover.*** Dysregulation in muscle protein turnover during estrogen
10 deficiency with the balance tipping away from protein synthesis and toward protein
11 degradation would contribute to fiber atrophy and thus overall loss of muscle mass. The
12 effects of estrogens on basal, resting skeletal muscle protein turnover have been studied in
13 women under various conditions of estrogen manipulation (for example in [26]) and
14 nominally in animal models.
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24 Muscle protein synthesis. Relatively more studies have measured rates of muscle
25 protein synthesis than degradation as related to estrogen status. Myofibrillar protein synthesis
26 at rest in pre-menopausal women did not differ between those in follicular (~low estrogen)
27 compared to luteal (relatively high estrogen) phase of their menstrual cycles [27]. A
28 shortcoming of this type of study design is that progesterone fluctuates in addition to estradiol
29 (the most bioactive estrogen in non-pregnant adult women) and therefore, delineating the
30 impact of estrogen versus progesterone on muscle protein synthesis is not possible. Studies in
31 which delivery of hormones is controlled circumvent this issue. Administering individual
32 hormones to post-menopausal women showed that progesterone alone increased the basal
33 rate of muscle protein synthesis but HT with estrogen alone had no significant effect [28].
34 Hansen and co-workers reported that myofibrillar rates of protein synthesis were lower in
35 post-menopausal women on estrogen-only HT compared to post-menopausal women not on
36 any HT [29]. In a separate study, those investigators reported that premenopausal women
37 taking oral contraceptive containing ethinyl estradiol also had a lower rate of myofibrillar
38 protein synthesis compared to young women not taking oral contraceptives [30].
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Another approach to assess estrogenic impact on rates of muscle protein synthesis is to compare rates between pre- and post-menopausal women. Two studies by Smith and coworkers showed that basal rates of muscle protein synthesis were 20-30% greater in post-menopausal compared to young, pre-menopausal women [28, 31], indicating that estrogen deficiency may improve the rate of protein synthesis. Overall, the collective evidence points in the direction of estrogens suppressing the rate of muscle protein synthesis in women, which is counterintuitive to the concept that estrogens will blunt the loss of skeletal muscle mass with age.

Studies using various animal models do not clarify the impact of estrogen on muscle protein synthesis. Skeletal muscle of ovariectomized rats induced to grow by reloading the hindlimbs after unloading had low levels of p70s6k and Akt compared to sham-operated rats indicating that estrogen deficiency impaired protein synthesis [32]. On the contrary, female lambs treated with a synthetic estrogen had decreased rates of muscle protein synthesis, despite the treatment causing increased growth [33]. Toth and coworkers utilized a rat ovariectomy model with hormone treatments and measured muscle protein synthesis rates [34]. Ovariectomized rats that received a placebo treatment had rates of protein synthesis that approximately doubled that in sham-operated, control rats as well as in ovariectomized rats that received estradiol or progesterone. These results parallel the overall human results of estrogen deficiency with muscle protein turnover appearing to tip toward anabolism rather than catabolism, as rates of protein synthesis tend to be greater in the absence of estrogen.

Muscle protein degradation. Smith and coworkers deduced that because skeletal muscle of post-menopausal women is in an overall catabolic condition, the relatively high rate of protein synthesis in the basal, resting state must be offset by an even greater rate of protein degradation resulting in a net protein and muscle loss [31]. However, whole body protein breakdown did not differ between aged, post-menopausal women not on HT

1 compared to young women with normal circulating estrogen [35], and myofibrillar protein
2 breakdown at rest did not differ between premenopausal women who did and did not take
3 estradiol-based HT [30]. Thus, results are insufficient to support the deduction that basal rates
4 of muscle protein degradation are elevated with estrogen deficiency.
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9 In animal studies, skeletal muscle protein degradation was not affected by estradiol
10 treatment in growing steers [36]. Though, using bovine satellite cell cultures, Kamanga-Sollo
11 and coworkers showed that estradiol treatment decreased the rate of protein degradation and
12 increased the rate of protein synthesis lending support to the concept of estrogen being
13 anabolic [37, 38].
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21 Turnover summary. Collectively, evidence that estrogen deficiency causes
22 dysregulation in muscle protein turnover with the balance tipping away from protein
23 synthesis and toward protein degradation and thus contributing to the loss of muscle mass is
24 weak. Further research, particularly on the protein degradation side of protein turnover, is
25 warranted. It is worth noting, that the evidence for estrogenic effects on protein synthesis and
26 degradation presented above focus on resting basal rates. There is some evidence for reduced
27 responsiveness of muscle protein synthesis to anabolic stimuli when estrogen is low [26].
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39 ***Muscle protein ubiquitination and proteasome activity.*** The ubiquitin-proteasome
40 system contains intracellular machinery for degrading and turning over proteins, with
41 ubiquitination having functions in addition to orchestrating proteolysis [39]. Protein
42 ubiquitination involves three classes of enzymes acting synergistically; ubiquitin activating
43 enzymes, ubiquitin conjugating enzymes and ubiquitin ligases. In addition, deubiquitinating
44 enzymes, the ubiquitin-specific peptidases (USPs), are needed for recycling ubiquitin and for
45 rescuing a protein from degradation. The ubiquitin-proteasome system is an important
46 mediator of skeletal muscle protein homeostasis and regulation of muscle mass through
47 crosstalk with anabolic and catabolic signaling pathways (reviewed by [40]). There has been
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little research to determine estrogenic impact on the function of the overall ubiquitin-proteasome system; what has been investigated has been directed mostly at ubiquitin ligases and peptidases.

The most prominent ubiquitin ligases in skeletal muscle are *atrogen-1* (official name Fbxo32, also known as MAFbx) and *MuRF1* (official name TRIM63, also known as MuRF2, IRF, RNF28), which were named as atrophy genes because their high expression leads to the loss of skeletal muscle mass whereas low expression generally leads to resistance against loss of muscle [41, 42]. The biology of ubiquitin ligases in skeletal muscle is complex as factors such as differences in muscle fiber types, acute versus gradual conditions inducing atrophy, and sex differences obscure the influence of these atrophy genes. For instance, an upregulation of *atrogen-1* and *MuRF1* genes was reported in atrophic muscles of aged male rats [43], while downregulation of *atrogen-1* and *MuRF1* was reported in muscles of aged female rats [44]. Such results of sex differences hint at a possible estrogenic effect, though the aged female rats were likely ovarian senescent and thus estrogen deficient. Rogers and co-workers more directly investigated estrogenic effects on ubiquitin ligases by comparing skeletal muscle from sham-operated and ovariectomized mice [45]. Muscle from ovariectomized mice had approximately 2-fold lower expression of *atrogen-1* and *MuRF1* compared to sham controls indicating that ubiquitin ligase-induced atrophy was suppressed when estrogen was lacking.

Svensson and coworkers performed a microarray study to investigate the effects of dihydrotestosterone, a non-aromatizable testosterone, and 17 β -estradiol on muscle gene expression in gonadectomized male mice [46]. As expected, gonadectomy resulted in reduced muscle mass that was partially reversed by estradiol and completely reversed by dihydrotestosterone treatment. Dihydrotestosterone and estradiol regulated mainly a different set of genes with only 13 genes regulated by both sex hormones. Among those dually

1 regulated was *atrogen-1*, which was upregulated in dihydrotestosterone- and estradiol-treated
2 mice despite muscle mass being preserved. No other ubiquitin-proteasome system genes were
3 identified in the study. Thus, the few reports on ubiquitin ligases in the rodent literature do
4 not provide support to the concept that estrogens may help maintain muscle mass. Rather,
5 many results are in opposition as estrogen deficiency was related to lower levels of atrophy
6 genes and estrogen-replete muscle had relatively high levels of atrophy genes.
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14 There is also a lack of consistent data in the human literature supporting the concept
15 that estrogen exerts beneficial effects to muscle by suppressing atrophy genes. Dieli-
16 Conwright and co-workers reported that postmenopausal women not on HT had lower muscle
17 mass, higher baseline muscle expression of *atrogen-1* and *MuRF1*, and smaller reductions in
18 the expression of these atrophy genes in response to a single bout of resistance exercise
19 compared to women on HT [47]. In contrast, Pöllänen and coworkers showed that muscle
20 biopsies from non-HT, early postmenopausal women engaged in a one-year long, randomized
21 HT trial had a higher number of differentially expressed ubiquitin-proteasome system genes,
22 including *atrogen-1*, compared to biopsies from HT-using women [48]. HT users had
23 upregulated *atrogen-1* expression concomitant with a gain of muscle mass while non-users
24 had reduced muscle mass over the year. Explanations for the seemingly contradicting results
25 between these two studies may include age of the participants, type of HT, and duration of
26 HT. In the latter study [48], women were relatively young (mean age 54 yr versus 59 yr in
27 [47]) and were randomized to a specific HT containing both estradiol and a progesterone,
28 whereas in the Dieli-Conwright study participants using either estrogen alone or estrogen plus
29 progesterone HT were recruited. Furthermore, baseline *atrogen-1* expression in the non-HT
30 participants did not change over the one-year follow-up period in the Pöllänen study, while in
31 the HT group there was 24% upregulation of *atrogen-1*. If the duration of HT were to
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continue for approximately 4 yr until study participants reached the same age range as in Dieli-Conwright study, it is unknown if *atrogen-1* expression would eventually decline.

Minimal investigation into estrogenic effects on ubiquitin-specific peptidases in skeletal muscle of women has been conducted. In the microarray study by Pöllänen, differential expression of four ubiquitin-specific peptidases (*usp1*, *usp2*, *usp15*, *usp50*) as well as one ubiquitin conjugating enzyme (*ube2g2*) in muscle between non-HT and HT postmenopausal women were reported [48]. Estrogen deficiency was related to upregulated expression of *usp1* and *usp15*, and downregulation of *usp2*, *usp50* and *ube2g2*, and real time RT-PCR validated these gene expression results, except for *usp50*. Any functional effects of estrogen on the deubiquitinating enzymes at the protein or activity level in muscle remain unknown.

A series of studies by Ogawa and co-workers investigated the role of estrogen in regulation of the ubiquitin-specific peptidase, USP19. Using a mouse immortalized skeletal myoblast cell line, C2C12, the group first showed that the level of USP19 protein was increased in a dose-dependent manner by estradiol [49]. Satellite cells isolated from mouse muscle and cultured in differentiation medium with and without estradiol also showed that protein and mRNA levels of USP19 were greater in the presence of estradiol. Consistent with the cellular results, USP19 protein was reduced after ovariectomy while a single intramuscular injection of estradiol restored the level back to the sham levels, which was shown to be mediated through estrogen receptor α [49]. The effect of USP19 was shown to be sex dependent with knockdown USP19 increasing muscle mass and fiber size in female but not male mouse soleus muscle [50]. Again, this was demonstrated to be mediated by estrogen receptor α as knockdown of the receptor caused a decrease in *usp19* gene expression and an increase in soleus muscle mass in female but not male mice. A third study demonstrated that USP19 is an estrogen-sensitive factor in the negative regulation of muscle

1 mass as estradiol treatment to ovariectomized mice resulted in higher soleus muscle USP19
2 gene and protein levels compared to those from untreated mice [51]. Collectively, the work
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4 by Ogawa and co-workers provides evidence that estrogen increases the expression of USP19
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6 and contributes to muscle atrophy. Another group reported no difference in muscle mass
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8 between female mice ablated for USP19 and control littermates [39]. These results are
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10 discrepant from Ogawa and coworkers, perhaps due to differences in mouse strains or the
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12 specific muscles studied.
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17 A last piece of evidence suggesting that the ubiquitin-proteasome system is involved
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19 in estrogen-mediated effects on skeletal muscle mass is that the system is important for the
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21 clearance of estrogen receptors, as these proteins carry conserved domains targeting them for
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23 ubiquitination-mediated degradation (reviewed by [52]). Because estrogens work primarily
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25 through estrogen receptors to mediate its effects, the turnover of estrogen receptors affects the
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27 transcriptional and signaling activity of estrogens.
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32 To summarize, the evidence that estrogen influences the ubiquitin-proteasome system
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34 in regulating skeletal muscle mass is scant and conflicting. Genes for ubiquitin ligases, the
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36 so-called atrophy genes, tend to be expressed at higher levels in muscle from estrogen-replete
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38 rodents, while data from the human literature are inconsistent. Similarly, gene expression of
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40 various ubiquitin-specific peptidases are differentially up- or down-regulated in estrogen-
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42 deficient muscle of women. The ubiquitin-specific peptidase, USP19, is relatively high in
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44 soleus muscle of estrogen-replete mice, which indicates that estrogen may contribute to
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46 muscle atrophy. The ubiquitin-proteasome system is very complicated with the genes and
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48 proteins discussed here for associations with estrogen representing only a small fraction of
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50 the total genes and protein. Thus, results from current research are insufficient to conclude
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52 how estrogen modulates the ubiquitin-proteasome system in skeletal muscle to contribute to
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54 the regulation of mass and strength.
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Skeletal muscle apoptosis. Biological aging is associated with increased cellular apoptosis leading to tissue dysfunction such as muscle atrophy. Estrogen is known to protect against apoptosis in non-skeletal muscle tissues [53-56]. Moreover, it has long been suggested that women are more protected from cellular damage (e.g., oxidative stress) that results in apoptosis; however, protection is lost following the menopausal transition [57].

Sexually dimorphic action of heat shock proteins in part regulate anti-apoptotic signaling in tissues [58, 59], including skeletal muscle [58-60]. In normal conditions, heat shock proteins are in low abundance but adequate for facilitating protein folding and acting as chaperones for newly synthesized proteins to their site of action. The loss of heat shock proteins results in mislocalized proteins, aggregation, protein breakdown, and unwanted protein-protein interactions. Following a stress event in skeletal muscle (e.g., exercise or damage), the intracellular heat shock response is activated and is necessary for the repair process. However, the heat shock response is blunted with age and can result in a chronic stress response and incomplete recovery of skeletal muscle [61].

There is evidence that heat shock proteins are estrogen sensitive in skeletal muscle [62-64]. A body of literature by Boland and Vasconsuelo and coworkers using C2C12 cells demonstrates that estrogen treatment protects the cells against hydrogen peroxide-induced apoptosis through upregulation of HSP27 [64-70]. HSP27 binds caspase-3, preventing its cleavage and keeping the enzyme in its inactive state, which in turn modulates its downstream targets, Bcl-2, BAD, AKT, ERK, and MAPK to block apoptosis and promote survival of C2C12 cells [64-70]. The few rodent studies that have been conducted show that estrogen treatment to males or ovariectomized females attenuates exercise-induced HSP70 and HSP72 (official name HspA1A) response and has no effect on HSP27 in hindlimb muscles [63, 71-73]. Wang and coworkers further showed that loss of estrogen in female rats blunted basal protein levels of HSP70, HSP27, and HSP90 in skeletal muscle [74]. Thus, it is

1 unclear how and which HSPs are regulated by estrogen; however, these studies provide
2 impetus for future research investigating estrogen's role in its protection against apoptosis
3 through HSP modulation especially in the complex *in vivo* environment of skeletal muscle.
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7 Only a few human studies to our knowledge have made measurements connecting
8 estrogen and apoptosis in skeletal muscle. As such, even less is known whether HSPs are
9 misregulated in skeletal muscle of post-menopausal women. Two studies utilized eccentric
10 exercise protocols to induce muscle damage in young, adult men and women [75, 76]. Each
11 study found men to have significantly higher basal levels of anti-apoptotic Bcl-2 in skeletal
12 muscle than women, whereas only women had increased Bcl-2 in muscle due to the eccentric
13 exercise. Kerksick and coworkers demonstrated greater change in cytoplasmic apoptotic
14 activity in response to damaging exercise in skeletal muscle biopsies of men than in women
15 [76]. These data, based on sex differences, suggest that estrogen may be protective against
16 skeletal muscle apoptosis in women.
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31 Further evidence supporting the regulation of apoptosis by estrogen comes from twin
32 studies on postmenopausal women discordant for the use of HT. In a study by Kangas and
33 coworkers, the expression of FasL, a ligand for cell death receptor Fas, and microRNAs miR-
34 21 and miR-146a known to target FasL and Fas [77, 78], respectively, were studied and
35 found to be less abundant in the serum samples of HT users compared to their non-using co-
36 twins [79]. Additionally, miR-146a in skeletal muscle was lower in the HT using twins.
37 While HSP's were not measured, miR-146a has been implicated in regulating the immune
38 response by targeting cytokine production, such as TNF- α and FasL [80]. These two
39 cytokines are part of the TNF family of death receptors and clustering these ligands and
40 receptors subsequently initiates signals for apoptosis [80]. Laakkonen et al also completed a
41 functional muscle proteomic analysis on biopsies from the twins and predicted that the non-
42 HT group had upregulated cell death and apoptotic pathways, as well as pathways related to
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1 the production of reactive oxygen species and necrosis [81]. The major cluster of
2 differentially expressed proteins indicated that the predicted upregulation of cell death
3 involves mitochondrial dysfunction occurring in women with low estrogen levels.
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7 Mitochondrial dysfunction has dual effects; it induces apoptosis and can also lead to
8 dysregulation of muscle energetic pathways, which in turn may contribute to muscle fiber
9 atrophy and eventually to the loss of muscle mass. Transgenic manipulation of estrogen
10 receptors has clearly demonstrated the importance of ER α -mediated estrogen signaling in
11 metabolic health [82-84]. Such studies have started to delineate the mechanisms underlying
12 the earlier observations pointing toward differences in signaling related to mitochondrial
13 function at the transcriptome [85, 86] and proteome [81] levels in skeletal muscle of
14 postmenopausal women. Muscle specific knockdown of ER α proved to have a critical role of
15 estrogen signaling in mitochondrial functions [82, 84]. However, due to the complexities of
16 increased fat and total body mass with estrogen receptor depletion [83, 87-90], it is difficult
17 to differentiate if the loss in muscle mass is the result of apoptosis induced by mitochondrial
18 dysfunction or as a side effect due to metabolic dysfunction in muscle.
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36 Accumulating evidence indicates that estrogen protects skeletal muscle from
37 apoptosis and thus defends against loss of muscle mass. However, the downstream, apoptotic
38 signaling pathways that respond to estrogen have yet to be conclusively determined.
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45 **2. Processes and players in muscle contraction affected by estrogen**

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47 Strength loss due to estrogen deficiency in women and female rodents cannot be fully
48 explained by loss of muscle mass because force generation that is normalized to muscle size
49 (also known as specific force) declines as well. Theoretically, an impairment in any step in
50 the process of muscle contraction, from propagation of an action potential at the motor end
51 plate to the calcium-activated interaction of actin and myosin in the sarcomere, can cause
52 diminished force generation. We are not aware of literature reporting estrogenic mechanisms
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in fiber excitation or excitation-contraction coupling of healthy muscle; rather the majority of investigations have focused on how estrogen affects sarcomeric proteins. Skeletal muscle incurs repetitive injury throughout life, requiring regeneration of the contractile machinery to regain strength. Here we will also review literature supporting the contention that regeneration of muscle is impaired when estrogen is lacking in females. The collective evidence presented indicates that dynapenia, due to aging or injury, is exacerbated by estrogen deficiency.

Myosin heavy chain. Phillips and co-workers first speculated that ovarian hormones influence muscle strength by directly affecting crossbridges, i.e., contractile proteins [8]. They showed that women did not differ from men in specific force of adductor pollicis muscle at young ages but past the age of menopause were weaker than men; HT, however, preserved strength in postmenopausal women. Wattanapermpool and Reiser more directly implicated contractile proteins when they reported that permeabilized fibers from ovariectomized rats produced less specific force than did fibers from ovary-intact rates [91]. Contraction is initiated in permeabilized fibers by direct application of calcium, thus only processes downstream of calcium release from the sarcoplasmic reticulum are engaged. Such results indicate that ovarian hormones affect a process between calcium binding to troponin C and the interaction of myosin heavy chain binding to actin and generating force.

Since that time, evidence continues to support the contention that estrogen affects myosin. Placing a spin probe on the catalytic domain of myosin heavy chain in fibers from mice with and without their ovaries, and then measuring strong-binding of myosin to actin during contraction by electron paramagnetic resonance spectroscopy, directly demonstrated that the generation of force at the molecular level was low with estrogen deficiency [92]. A subsequent study showed that estradiol treatment to ovariectomized mice could prevent and restore loss of strong-binding of myosin to actin and force generation [93]. Active stiffness is

1 an indirect estimate of myosin bound to actin during contraction and results using this
2 measure are consistent with estrogen exerting a positive influence on myosin heavy chain
3 function [93, 94]. Specific force of isolated mouse muscles in these studies were also greater
4 in estrogen-replete compared to α -deficient mice [92-95] and the estrogenic effects are
5 mediated through estrogen receptors [96].
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11 A study of permeabilized single muscle fibers from post-menopausal twins provides
12 further convincing evidence that estrogen affects the function of myosin heavy chain. In this
13 study by Qaisar and coworkers, specific force and myosin function, as determined by force
14 per crossbridge, was greater in fibers from biopsies of sisters on HT compared to sisters not
15 on HT [97].
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24 Myosin heavy chain is an ATPase and recently a novel regulated state of this protein
25 characterized by extremely slow ATP turnover during relaxation (i.e., not during contraction)
26 was discovered and termed the super-relaxed state (SRX) [98]. Age-related changes in
27 myosin SRX is specific to females [99] and is regulated at least in part by estradiol [100].
28 Downstream mechanisms underlying estrogenic effects on myosin function during relaxation
29 are speculated to be related to phosphorylation of myosin's regulatory light chain [99, 100].
30 Further studies are required to elucidate the extent to which the state of myosin during
31 relaxation translates to modulation of force generation during contraction and thus strength in
32 females.
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46 ***Myosin regulatory light chain.*** Myosin consists of two heavy chains and two pairs of
47 light chains that are stabilized on the thick filament by myosin binding protein C. The
48 regulatory light chain (RLC) acts as a lever arm on the α -helical neck region of the myosin
49 heavy chain, transmitting the free energy of ATP hydrolysis down the neck domain to
50 amplify movements of myosin and force production during contraction [101]. In skeletal
51 muscle, RLC phosphorylation modulates myosin structure, force, and power output [102,
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103] and can influence contractile strength during daily tasks [104]. Estrogen has been implicated in the regulation of RLC phosphorylation and contractility of cardiomyocytes [105]. In skeletal muscle, proteomic studies showed age-dependent reduction of RLC phosphorylation in human vastus lateralis muscle [106] and in rat fast-twitch muscle [107]. Muscle biopsies from young and older men and women also showed that phosphorylation of RLC was reduced in fibers of old, estrogen-deficient women but not old men, relative to young counterparts [108]. Such results implicate estrogenic impact on post-translational modification of myosin RLC.

Direct evidence that estrogen modulates phosphorylation of RLC in skeletal muscle comes from the work of Lai and coworkers [109]. Estradiol treatment increased RLC phosphorylation in C2C12 cells, as well as in skeletal muscles of ovariectomized mice [109, 110]. The estradiol-induced phosphorylation of RLC in female mice was related to force generation as measured by posttetanic potentiation of force [109]. Several other skeletal muscle proteins including myosin binding protein C, myosin essential light chain, troponin I, titin, and nebulin, affect contractility and/or myosin structure when phosphorylated, but whether or not those proteins are modulated by estrogen await additional studies.

The collective evidence supports the view that estrogen enhances muscle strength by affecting the function of myosin, specifically myosin through phosphorylation of the RLC. Future research to identify estrogenic effects on other sarcomeric proteins are warranted, as is determination of estrogen-sensitive kinases that phosphorylate sarcomeric proteins.

Impaired regeneration following injury. The concept that repeated skeletal muscle injury followed by inadequate repair contributes to muscle weakness in the aged has been put forth [111-113]. Several lines of evidence indicate that estrogen affects the recovery of muscle following injury and thus, estrogen deficiency in females would theoretically further exacerbate muscle weakness.

1 The best indicator of recovery from injury is the capacity of skeletal muscle to
2 produce force, that is, regain strength [114]. Ovarian hormones have been implicated in the
3 recovery of strength in females because young, adult mice that are ovariectomized have an
4 incomplete recovery of strength following contraction-induced injury [115] and estradiol
5 treatment improves recovery of strength following a traumatic freeze injury [116]. Consistent
6 but less direct evidence for a role of estrogen comes from two studies on aged mice by Rader
7 and Faulkner. Results from those studies demonstrate that strength recovery after eccentric
8 contractions is substantially worse in 25-29 month-old females [117], which are presumably
9 ovarian senescent and thus estrogen deficient, compared to aged males [118].
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22 Mechanistically, it has been hypothesized that regulation of muscle inflammation and
23 satellite cell function are perturbed by the loss of estrogen and may contribute to impaired
24 strength recovery. Although the effects of estrogen on the inflammatory response following
25 injury has been reported in a fair number of studies on females, the results are conflicting.
26 The effects of estrogen on muscle inflammation in studies on women are inconsistent with
27 evidence that estrogen decreases [119], increases [120], or does not affect [121] inflammation
28 following muscle injury. Using rodent models, studies show that estrogen treatment
29 attenuates [122-125], enhances [120, 126-128], or has no effect [129, 130] on muscle
30 inflammation. A recent study showed that a moderate, physiological dose of estradiol given
31 to ovariectomized mice increased the inflammatory response via recruitment of neutrophils
32 following muscle injury and also demonstrated that recovery of strength was enhanced
33 compared to a placebo treatment [116]. It was further proposed that the serum level of
34 estrogen is a critical component that needs to be considered in future research because
35 supraphysiological levels of estrogen may elicit opposite inflammatory effects than
36 physiological levels, contributing to inconsistencies in the literature, as serum estradiol levels
37 are often not reliably measured in rodent studies.
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1 The inflammatory response following skeletal muscle injury subsequently affects the
2 myogenic response during regeneration (Reviewed by [131]). Studies from Tiidus and
3 colleagues indicate that exercise-induced activation of satellite cells is less effective in the
4 absence of estrogen [132, 133] and this response appears to be mediated by estrogen
5 receptors [130, 134]. Similarly, cell culture studies have shown an estrogen dose-dependent
6 increase in myogenic cell proliferation [135]. While, the data in humans is sparse, myogenic
7 regulatory factor gene expression is acutely increased following eccentric exercise in post-
8 menopausal women on HT compared to those not on HT [47].
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19 Current evidence supports the premise that estrogen affects the regeneration of
20 skeletal muscle by influencing the inflammatory and myogenic responses, although the
21 underlying mechanisms are largely unknown and await future research. Studies with more
22 controlled methods of estrogen treatment and reliable serum measurements, robust markers of
23 inflammatory and satellite cell functions, and details of how those processes ultimately affect
24 recovery of muscle strength are needed.
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36 **Conclusions**

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39 The loss of muscle strength in females due to estrogen deficiency results from both
40 inadequate preservation of skeletal muscle mass and quality of the remaining skeletal muscle
41 (Figure 1). Evidence that estrogen impacts muscle protein turnover and the ubiquitin-
42 proteasome system is overall weak. There is stronger evidence that estrogen protects skeletal
43 muscle against apoptosis via effects on HSPs and mitochondria. Thus, when estrogen is
44 deficient it appears that apoptotic pathways contribute to the loss of muscle mass. The
45 leading candidate causing dynapenia due to estrogen deficiency in females is myosin.
46 Modifications to myosin heavy chain function through phosphorylation of myosin RLC have
47 been demonstrated in both female rodent models and women. Also contributing to the loss of
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1 muscle strength in females is aberrant inflammatory and satellite cell responses when
2 estrogen is deficient. Delving deeper into the mechanisms underlying estrogenic effects on
3 skeletal muscle apoptosis, satellite cells, myosin, and overall signaling pathways will further
4 inform scientists and clinicians designing therapeutic strategies to combat sarcopenia and
5 dynapenia.
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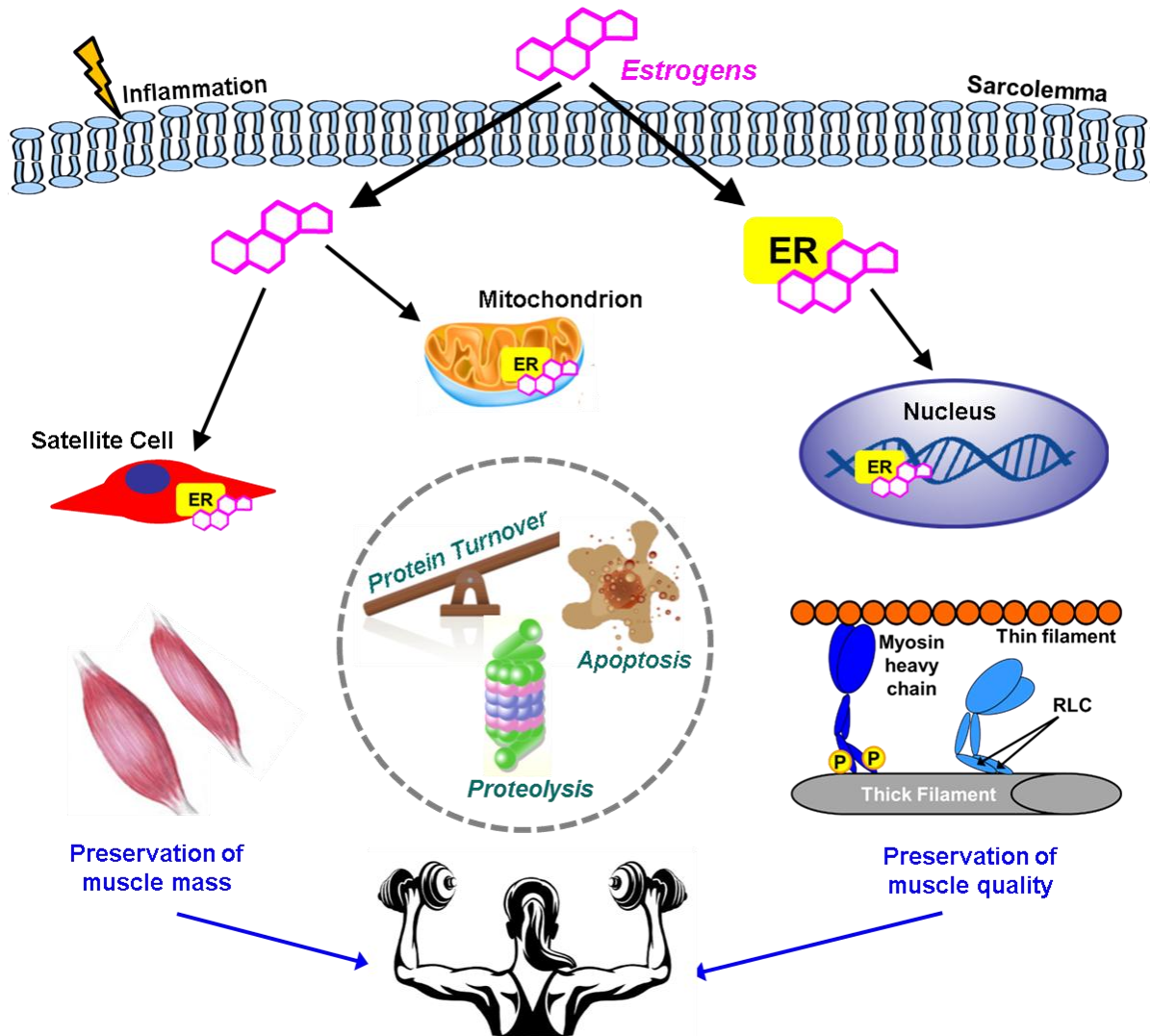


Figure Legend

Figure 1. Estrogens affect skeletal muscle strength in females by preserving muscle mass and quality of the contractile proteins. Estrogens influence the binding of myosin heavy chain to actin to generate force through phosphorylation of the regulatory light chain. Processes that are impacted by estrogens affecting muscle mass may include protein turnover, proteolysis, and apoptosis. Organelles that are sensitive to estrogens and contribute to maintenance of muscle mass and quality include nuclei and mitochondria. Inflammation and satellite cell function are also estrogen sensitive and can play a role in ultimately preserving muscle strength. ER = estrogen receptor; P = phosphorylation; RLC = regulatory light chain

Acknowledgements

This work was supported by National Institutes of Health grants R01-AG031743 (DAL) and T32-DK091317 (BCC), and the Academy of Finland (309504; EKL). We thank Georgios Kararigas for critical reading of the manuscript.

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