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Estradiol treatment, physical activity, and muscle function in ovarian-senescent mice

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Abstract

Estradiol (E_2) treatment in young adult, ovariectomized mice increases physical activity and reverses deleterious effects on skeletal muscle. Here we test the hypothesis that E_2 treatment improves muscle function and physical activity in aged, ovarian-senescent mice. Plasma E_2 levels and vaginal cytology confirmed ovarian senescence in 20-month-old C57BL/6 mice. Mice were then randomly divided into activity groups, having access to a running wheel or not, and further into those receiving E_2 or placebo. Placebo-treated mice wheel ran more than E_2 -treated mice ($P=0.03$), with no difference between treatment groups in cage activities such as time spent being active and ambulation distance ($P\geq 0.55$). Soleus muscles from aged mice that wheel ran adapted by getting larger and stronger, irrespective of E_2 status ($P\leq 0.02$). Soleus muscle fatigue resistance was greater in mice treated with E_2 ($P=0.02$), but maximal isometric tetanic force was not affected ($P\geq 0.79$). Because E_2 treatment did not improve physical activity or overall muscle function in the aged, ovarian-senescent mice as predicted, a second study was initiated to examine E_2 treatment of young adult mice prematurely ovarian senescent from exposure to the chemical, 4-vinylcyclohexene diepoxide (VCD). 4-month-old C57BL/6 female mice were dosed with oil (control) or VCD. Vaginal cytology confirmed ovarian senescence in all mice treated with VCD 63 days after the onset of dosing, and then a subset of the VCD mice received E_2 (VCD+ E_2). Wheel running distance did not differ among control, VCD, and VCD+ E_2 mice ($P\geq 0.34$). Soleus muscle concentric, isometric, and eccentric *in vitro* forces were greater in VCD+ E_2 than VCD mice ($P<0.04$), indicating beneficial estrogenic effects on muscle function. In general, aged and young mice with senescent ovaries were less responsive to E_2 treatment, in terms of physical activities and muscle function, than what has previously been shown for young, ovariectomized mice. These results bring forth the possibility that some component of the residual, follicle-depleted ovarian tissue influences physical activity in mice or that aging diminishes the responsiveness of skeletal muscle and related tissues to E_2 treatment.

Keywords

4-vinylcyclohexene diepoxide; aging; exercise; skeletal muscle

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1. Introduction

Aging results in many structural, functional, and biochemical changes that can impact the ability or the willingness to do physical activity [1]. This scenario is complicated in females because changes in ovarian hormones occur simultaneously with aging and some of the tissues important for regulating and doing physical activity are affected by both age and ovarian hormones. For example, in rodents the hypothalamic areas that regulate physical activity are affected by both aging and alterations in estrogen status [2,3,4]. Similarly, skeletal muscle weakness occurs with age and there is evidence for a greater decline in strength at the time of menopause in women [5] and ovarian failure in female mice [6]. One approach for dissecting out the influences of aging versus ovarian hormones is to ovariectomize young female rodents. Although this is closer to a model of surgical menopause in young women, it is advantageous because the effects of ovarian hormones can be assessed independent of age. Using this approach, physical activity in terms of voluntary wheel running decreases by as much as 90% and cage activities are reduced by about 50% in young female rodents post-surgery [7,8,9]. Similarly, skeletal muscle force generation decreases by 10 to 20% post-surgery [10,11,12]. Furthermore, it appears that estrogens are the primary hormones responsible for the decreases, as 17 β -estradiol (E₂) treatment reverses these ovariectomy-induced physical inactivities within a few days [7,8,9,13] and skeletal muscle function can be restored in as early as 14 days [7,11,12]. Similarly, female rodents become less active with age [1], but whether or not low circulating estrogen levels contribute to this age-reported physical inactivity is not known. Thus, the first purpose of this study was to determine the effects of E₂ treatment on physical activity of older female mice that had experienced natural, age-induced ovarian senescence. Physical activity was monitored by both voluntary wheel running and several parameters of daily cage activities. We hypothesized that physical activities would be greater in aged, ovarian-senescent mice treated with E₂ compared with those not treated.

It is unclear if exercise-induced skeletal muscle adaptations differ for post-menopausal women on estrogen-based hormone therapy compared to those not. Some studies have shown no differences between interventions of exercise alone or exercise in combination with estrogen-based hormone therapy [14,15,16], while others have shown additive effects of exercise and hormone therapy [17,18]. Beneficial skeletal muscle adaptations do occur in response to both physical activity and estradiol treatment in young, ovariectomized mice [7,11,12]. As such, a second purpose of this study was to determine the extent to which muscle of aged, ovarian-senescent mice adapt to physical activity and to E₂ treatment. We hypothesized that mice engaged in physical activity would have better soleus muscle contractility compared with those that were relatively sedentary, and soleus muscle contractile function would be greater in aged, ovarian-senescent mice treated with E₂ versus those that remained untreated. Specifically, we hypothesized that maximal isometric force and active stiffness would be greater in soleus muscles of aged, ovarian-senescent mice treated with E₂ compared with those not treated. Furthermore, because there have been sex implications in resistance to muscle fatigue [19], we hypothesized that there would be a protective effect of E₂ on soleus muscle fatigability.

Our initial hypotheses were not supported, as the aged, ovarian-senescent mice treated with E₂ did not have improved physical activities or overall muscle function compared to the placebo-treated, aged mice. Thus, the E₂ benefits on physical activity and skeletal muscle observed in young ovariectomized mice might be influenced by the absence of the ovaries. Alternatively, those tissues important for doing or regulating physical activity and muscle contractility are responsive to E₂ treatment in young but not aged mice. We addressed these two possibilities using a mouse model of premature ovarian senescence allowing us to investigate senescent ovaries in otherwise young mice [20,21]. Chemical dosing of 4-

vinylcyclohexene diepoxide (VCD) accelerates the normal process of apoptosis and atresia of ovarian follicles resulting in state of persistent diestrus and low circulating E₂ levels, similar to what occurs with age in rodents [22,23]. The advantages of this approach is that young adult mice can be utilized to study the influences of ovarian hormones without the confounding effects of advanced age, similar to ovariectomy, but while having the ovarian tissue remain intact. Furthermore, the hormone profile of the follicle-depleted, ovary-intact young mouse after VCD treatment is similar to the aged, ovarian-senescent mouse as opposed to the abrupt changes in hormones that occur with ovariectomy [24,25]. Specifically, with VCD exposure, follicle stimulating and luteinizing hormones gradually increase, while progesterone, androstenedione, and E₂ progressively decrease to levels found in age-induced, ovarian senescence mice [23,25]. VCD treatment has been shown to specifically target the ovary with no effects on other tissues [26,27,28,29,30,31,32].

Using young adult, VCD-treated mice with and without subsequent E₂ treatment, we tested the hypothesis that physical activity and soleus muscle function would be reduced when circulating E₂ was diminished and preserved when E₂ was restored. Support of this hypothesis would lead us to believe that indeed E₂ is a key ovarian hormone for physical activity and skeletal muscle function and that perhaps in our previous study using aged mice the dosing regimen was not optimal or the aged mouse was not able to respond to the E₂ treatment.

2. Methods

2.1 Animals

Female C57BL/6 mice (n = 60) were purchased through the National Institute of Aging at 17 months of age and then given 1 week to acclimate to our facility upon arrival for Study I. Twelve of the initial 60 mice were not used in the study. Six of the mice were excluded before being assigned to a group; three had skin irritations in the neck area that would have compromised pellet implantation, one had an abdominal tumor, and two died of unknown causes. The other six mice were eliminated during the study due to bacterial infection (n=1), inner ear infection (n=1), distended urinary bladder (n=1), and death of undetermined cause (n=3).

A second set of female C57BL/6 mice (n=24) were purchased from Jackson Laboratories (Bar Harbor, ME) at 3 months of age for Study II. These mice were housed at the Jackson In Vivo Laboratory Facility and were dosed daily for 15 days with either sesame oil (n=8) or VCD (n=16) via intraperitoneal injections at a dose of 160 mg/kg body mass/day [23]. Five of the VCD mice died during treatment at Jackson Laboratories; technical reports indicated the mice appeared fine except for abnormal gait patterns following their last injection prior to death. Thus, a total of nineteen, 4-month-old mice arrived in our facilities for Study II.

Aged mice in Study I were housed five per cage until persistent diestrus was confirmed and at that time were placed in individual cages. Mice in Study II were group housed for the duration of the study. All mice were maintained on a 12-hr light cycle and had free access to water and a phytoestrogen-free chow (2019 Teklad Global 19% Protein Rodent Diet, Harland Teklad, Madison, WI). This chow was used to eliminate potential complications from exogenous forms of estrogens.

At the end of each study, when mice were ~22- and 8-months of age for Study I and II respectively, mice were weighed and anesthetized by intraperitoneal injection of sodium pentobarbital (100 mg/kg body weight), with supplemental doses given as needed prior to *in vitro* muscle contractile function testing. After muscles and uteri were harvested, mice were euthanized by an overdose of sodium pentobarbital (200 mg/kg body weight). All protocols

were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

2.2 Experimental design of Study I

Ovarian senescence was determined by vaginal cytology. Cytology was assessed 2–3 times per week and a mouse was deemed to be in persistent diestrus when two consecutive samples showed predominantly leukocytes [33]. Of the 60 mice initially received, 7 were in persistent diestrus upon arrival. By 18- and 19-months of age, 10 and 19 mice entered persistent diestrus, respectively. In all, mice had failed ovaries by an average of 19.7 ± 0.2 (mean $\pm$ SE) months of age. This was confirmed by the low plasma levels of E_2 one week prior to the onset of the study; 38 of the 40 mice that were tested had values below the assay detection level (10 pg/ml) and the remaining 2 mice averaged 15.2 ± 0.4 pg/ml. Because the timing of E_2 treatment following hormone deficiency is critical [34,35], within 2–4 weeks of when each mouse entered persistent diestrus, the following study design was initiated (Figure 1).

Approximately half of the mice were given running wheels for 1 week in their home cages for acclimation purposes. Distance run during the last 3 days was used to rank mice according to running performance. These mice were divided into two groups based on a rank-order scheme designed to minimize baseline differences between the two groups in wheel running performance. The mice then received pellet implants, either E_2 (run- E_2 ; $n=12$) or placebo (run-placebo; $n=9$). Wheels were taken out of cages for 3 days to allow recovery from the minor pellet-implantation surgery. Wheels were then reintroduced and voluntary running continued for approximately 8 weeks. Mice that did not wheel run, i.e., remained sedentary, were also subdivided into two groups and received pellets; either E_2 (sed- E_2 ; $n=10$) or placebo (sed- placebo; $n=9$) and remained in their individual cages for ~8 week. 24-hr cage activity measurements were conducted on all mice during week 7 of the hormone manipulation period. Body mass of each mouse was measured weekly and food intake was monitored daily throughout the study. At ~22 months of age each mouse was anesthetized and soleus muscles underwent *in vitro* contractile function testing.

Eight additional 17 month-old mice not included in the previous study design were used to investigate the influence of the ovarian tissue on wheel running. Once persistent diestrus was confirmed, all mice were given running wheels for 1 week. The mice were then randomized and underwent either a sham ($n=3$) or ovariectomy ($n=5$) procedure. Following ~1 week of recovery mice were re-introduced to running wheels for 2 weeks.

2.3 Experimental design of Study II

VCD-treated mice were assessed daily for estrous cycle stage until persistent diestrus and thus ovarian senescence was confirmed. VCD mice were considered acyclic after 10 days of diestrus [36], which occurred 63 ± 3 days following the onset of VCD dosing. Once persistent diestrus was confirmed, each mouse underwent physical activity testing including wheel running and cage activities. Then, ~4 weeks later (when mice were ~6 months of age), hormone manipulation began and lasted for 8 weeks; experimental groups were VCD ($n=5$), VCD+ E_2 ($n=6$), and control ($n=8$) (see Figure 1). The control group consisted of the mice that had received sesame oil injections at Jackson Labs. During the 8-week intervention, mice underwent assessments of wheel running motivation (during weeks 2 and 5), cage activities and food intake (during weeks 1 and 7), and weekly body mass. At the end of 8-week intervention, mice were ~8 months of age, and were anesthetized and soleus muscles underwent *in vitro* contractile function testing.

2.4 Hormone manipulation and related procedures

Mice were anesthetized in an induction chamber with isoflurane and then maintained via inhalation of 1.75% isoflurane mixed with oxygen at a flow rate of 200 ml/min delivered via a mask. Once anesthetized, mice were implanted with a 60-day slow-release E₂ or placebo pellet (Innovative Research of America, Sarasota, FL) on the dorsal aspect of the neck using a trochar. In Study I, E₂ pellets contained 0.09 mg of 17 β -estradiol, delivering ~1.5 μ g daily. This dosage was used for two reasons. First, a dosage was desired that would mimic the average circulating level of E₂ in young, adult female mice over the estrous cycle, i.e., ~15 pg/ml serum [37]. In a preliminary study using these pellets we measured plasma E₂ from four mice at 16.01 ± 5.3 pg/ml. Second, we also found in preliminary studies that 0.18 mg 17 β -estradiol pellets caused urine retention in aged mice, which has been reported by others as well [38,39,40]. Bladder problems were minimized by using the lower dose pellets as only one mouse was excluded due to a distended urinary bladder. In Study II, a subset of the VCD-treated mice were implanted with 0.18 mg, 60-day slow-release E₂ pellets, which delivers ~3 μ g per day. We used the higher dose pellet for these younger mice because we desired to mimic our previous experiments on young ovariectomized mice [7,9,11]. Placebo pellets used in both studies consisted of the same matrix as the E₂ pellets.

Ovariectomy of mice was conducted as previously described [7,10,11]. While under isoflurane anesthesia the ovaries were removed bilaterally. Mice undergoing the ovariectomy procedure were administered 0.15 μ g of buprenorphine subcutaneously ~5 minutes post procedure. Blood was collected by facial vein bleed, plasma was separated from whole blood, and E₂ was measured by ELISA (KA0234, Abnova Corporation, Taiwan). The uteri were dissected and immediately weighed as additional marker of ovarian-hormone depletion and hormone treatment.

2.5 Physical activity assessments

Voluntary wheel running was measured using an exercise wheel, 11 cm in diameter with a 2 inch wide running surface, that was mounted to the top of a standard mouse cage [9]. A digital magnetic counter was fastened to the wheel and connected to a microprocessor which stored the number of revolutions per 24 hr. In Study II, mice were allowed access to the wheels on three separate 3-day periods to assess physical activity without any anticipated training effects [41].

Cage activities were evaluated by first placing individual mice in a mock activity chamber to acclimate to the new environment for 24 hr immediately prior to the test. Mice were then placed individually into an activity chamber (Med Associates Inc., St. Albans, Vermont) for 24 hr [7,42]. Data presented are total active time, jumping counts, rearing counts, and ambulatory distance and were collected using Activity Monitor version 6.01 software (Med Associates) with a box size set to “3” (4.8 cm²). While in the activity chamber, food intake was also measured for each mouse in Study II.

2.6 In vitro assessment of soleus muscle contractility

The dissection and testing protocol for the isolated soleus muscle preparation has been described previously [6,43]. First, passive muscle stiffness was determined by stretching inactive muscle sinusoidally from 97.5% L₀ to 102.5% L₀ at 0.5 Hz and measuring the force response (300B-LR; Aurora Scientific Inc., Aurora, ON, Canada). Maximal isometric twitch force (P_t) was then elicited by stimulating the muscle with a 0.5-ms pulse at 150 V (Grass S48 stimulator delivered through a SIU5D stimulus isolation unit; Grass Telefactor, Warwick, RI) and maximal isometric tetanic force (P₀) determined by stimulating muscles for 400 ms at 150 V and 120 Hz. Active stiffness was determined during a second maximal tetanic contraction by eliciting a sinusoidal length oscillation of 0.01% L₀ at 500 Hz.

Additional parameters measured from twitch and tetanic contractions include; time to peak twitch (TPT), twitch one-half relaxation time ($RT_{1/2}$), maximal rate of tetanic force development ($+dP/dt$), and maximal rate of relaxation ($-dP/dt$).

Soleus muscles from mice in Study I then underwent a protocol employing a bout of fatiguing contractions that began 2 min following the final force and stiffness measurements [7]. For soleus muscles from Study II, they then were measured for maximal concentric force that was elicited by passively lengthening the muscle to 105% of L_0 over 3 s, and then stimulating tetanically for 133 ms as the muscle was shortened to 95% of L_0 at 1.5 L_0/s . Finally, eccentric force was elicited by passively shortening the muscle to 95% of L_0 and then stimulating tetanically as it lengthened to 105% of L_0 at 1.5 L_0/s .

Following contractility testing, each soleus muscle was removed from the bath, trimmed at the myotendinous junctions, blotted, weighed, and stored at -80° until further analysis. Fiber length was estimated as 71% of the soleus muscle length [44]. Specific P_0 was calculated by correcting for physiological cross-sectional area of the muscle [45].

2.7 Statistical analysis

In Study I, a two-way repeated measure ANOVA was used to determine if differences existed for wheel-running distances between mice treated and not treated with E_2 over time. Student t-tests were used to examine possible differences in cage activities between mice treated and not treated with E_2 . A three-way repeated measure ANOVA (treatment $\times$ activity level $\times$ time) was used to determine differences in food intake. Two-way ANOVAs (treatment (E_2 or placebo) $\times$ activity level (wheel runners or sedentary)) were used to determine if there were differences among the four groups for body mass, organ masses, muscle composition, and soleus muscle contractility measures. Holm-Sidak post-hoc tests were applied whenever a significant interaction or main effect was detected by ANOVA.

For Study II, two-way repeated measures ANOVAs (group $\times$ time) were conducted to assess body mass, activity and running. One-way ANOVAs were used to determine differences among groups for food intake, muscle contractile parameters, uterine masses, and muscle masses. Holm-Sidak post-hoc tests were applied whenever a significant interaction or main effect was detected by ANOVA.

Statistical analyses were done using SigmaStat version 3.5 (Systat Software Inc; Point Richmond, CA), with the exception for the three-way repeated measures ANOVA, which was conducted using JMP version 7.0 (SAS Institute, Inc; Cary NC). Significance was accepted with an α level of 0.05. Values are reported as means $\pm$ SE.

3. Results

3.1 Study I: Ovarian senescence in aged mice

Body mass was not different among groups at the beginning or end of the 8-week study (i.e., when mice were 20 and 22 months old, respectively), although there were trends for E_2 -treated mice to have lower body masses than those treated with placebo (Table 1). On average, mice ate 4.1 ± 0.1 g of food per 24 hr with no effect of hormone status or physical activity ($P>0.457$). Mice treated with E_2 had uterine masses nearly twice that of placebo mice indicating effective E_2 treatment (Table 1).

3.1.1 Physical activities—Of the non-wheel running mice, there was no difference between those treated with placebo and E_2 for any parameter of daily cage activity. Mice were active for about 4 hr per day, 234 ± 31 vs. 248 ± 12 min for placebo and E_2 mice, respectively ($P=0.555$). Distance ambulated per 24 hr was not different between groups, 415

± 50 vs. 365 ± 72 m for placebo and E_2 mice, respectively ($P=0.608$). During the active periods, placebo- and E_2 -treated mice had equivalent counts for jumping and rearing activities as well ($P \geq 0.551$).

Mice that had access to running wheels were affected by E_2 as far as distances run. Prior to receiving placebo or E_2 treatments, mice ran an average 2.8 ± 0.3 km per 24 hr (Figure 2). There was an effect of both time and treatment on running distances with the E_2 -treated mice running significantly less than placebo-treated mice during the 8-week study ($P \leq 0.027$; Figure 2).

To begin to investigate discrepancies of E_2 effects on physical activities between previously-reported young, ovariectomized mice and aged, ovarian-senescent mice in these studies, an additional set of 8 aged, ovarian-senescent female mice were studied. Once persistent diestrus was confirmed, mice were randomized to a surgical group, either a sham surgery or ovariectomy. However, before any surgery took place mice wheel ran for 1 week and averaged 4.5 ± 0.4 km per 24 hr (Figure 3). Mice were then subjected to surgery. During the next 2 weeks, the sham-operated, ovary-intact mice ran 3.5 ± 0.6 km per 24 hr while the ovariectomized mice ran only 1.9 ± 0.4 km ($P=0.012$; Figure 3). These data suggest that the presence of residual, ovarian tissue influences wheel running in aged mice.

3.1.2 Muscle contractility—Soleus muscles from aged, ovarian-senescent mice that ran on wheels for ~8 week had greater capacity to generate force than those without running wheels, irrespective of E_2 treatment. Specifically, soleus muscles of running mice had 21 to 23% greater mass, P_0 , and active stiffness (Table 2). Examination of force-time tracings revealed no effect of either wheel running or E_2 on time to peak twitch (TPT), twitch one-half relaxation time ($RT_{1/2}$), or tetanic rates of contraction ($+dP/dt$) or relaxation ($-dP/dt$) (Table 2). E_2 treatment of the aged, ovarian-senescent mice affected the fatigability of the soleus muscle, but no other contractile parameter (Table 2). With the 60-contraction fatiguing bout, there was a main effect of E_2 on fatigability from the 10th contraction onward ($P \leq 0.032$; Figure 4). Specifically, E_2 was protective against soleus muscle fatigue with ~10% greater fatigue resistance in E_2 -treated mice, irrespective of wheel running. The force decrement was confirmed to be fatigue as opposed to muscle injury as P_0 5 min following the fatigue protocol (post fatigue) was not different than P_0 pre-fatigue in any of the four groups of mice ($P \geq 0.229$; Figure 4 insert).

3.2 Study II: Premature ovarian senescence in adult mice

Body mass did not differ between the control, VCD, and VCD+ E_2 groups of mice (main effect of group, $P=0.251$), but was affected by time. Initially mice weighed 23.0 ± 0.3 g and at the end of the 8-week study mice weighed 25.1 ± 0.4 g (main effect of time, $P < 0.001$). Mice ate an average of 3.4 ± 0.1 g of food per 24 hr with no effect of group ($P=0.485$). Periodic vaginal cytology confirmed that control mice were cycling normally and that VCD and E_2 interventions were effective throughout the 8-week study. At the end of the study, control mice had uterine weights different than VCD and VCD+ E_2 -groups, indicating that both the VCD and E_2 -treatments were effective in atrophy and hypertrophy the uterus, respectively (118.2 ± 13.0 , 50.4 ± 7.6 , and 131.9 ± 27.5 g for control, VCD, and VCD+ E_2 , respectively; $P=0.021$).

3.2.1 Physical activities—Acute wheel running and 24-hr cage activity were measured three times during the study as indicators of physical activity. There was no difference in running among groups and only moderate differences in cage activities (Table 3). Total active time increased by 1–2 hr per day over the 8-week study for the VCD+ E_2 mice and these mice were more active for ~1.5 hr per day than were control mice at week 7 (Table 3).

There were no significant differences in ambulatory distance among groups and rearing counts increased over time irrespective of group (Table 3).

3.2.2 Muscle contractility—There was no difference among groups in soleus muscle size (Table 4). However, mice that were prematurely ovarian-senescent due to VCD and then replaced with E₂ for 8 weeks had greater soleus muscle strength than VCD mice not treated. Soleus muscles from VCD+E₂ mice generated 16–19% more concentric, isometric, and eccentric force than VCD mice (Figure 5; $P \leq 0.039$). This was also evident in concentric and isometric specific forces which were 10–12% greater in VCD+E₂ relative to VCD mice ($P \leq 0.009$), but eccentric specific force did not differ among groups ($P = 0.218$). There were no differences among groups in passive stiffness or peak twitch force (Table 4). Active stiffness, indicative of strong-binding myosin to actin, showed a trend for the soleus muscles from VCD+E₂ mice to be greater (Table 4). Force-time tracings of twitch and tetanic contractions revealed minimal effects of VCD or E₂ treatment on rates of muscle contraction and relaxation with only tetanic $+dP/dt$ being faster in the VCD+E₂ than the VCD group of mice (Table 4).

4. Discussion

The overall results of these studies indicate that physical activity and skeletal muscle function in mice that are ovarian-senescent, either due to age or chemical treatment, respond to E₂ treatment somewhat differently than what has previously been shown in young, ovariectomized mice. When young mice are ovariectomized physical activities plummet by as much as 90% [7,8] and muscle force generation diminishes ~10% [46]. When these mice are replaced with E₂ there are increases in voluntary wheel running and physical activities [7,9,47], and reversal of ovarian hormone-induced strength losses [7,11,12,48] back to pre-surgery levels. In the first study reported here, we investigated physical activity and soleus muscle contractile function in 22-month-old, ovarian-senescent mice and found that 8 weeks of E₂ treatment in those mice did not affect cage activities, decreased wheel running, and had no affect on soleus muscle force generation. These conflicting results of E₂ treatment in estrogen-deficient young and aged mice prompted us to design a second study to examine the influence of the presence of failed, follicle-depleted ovaries in a young, 8-month-old mouse model of premature ovarian senescence. In these young mice, our primary findings were that physical activities were minimally altered by inducing ovarian senescence or by E₂ treatment. Our hypotheses regarding soleus muscle function were partially supported as premature ovarian senescence did not result in reduced soleus muscle force production but greater forces were generated in response to E₂ treatment. Collectively, the results of these two studies indicate that ovarian-senescent mice are less responsive to E₂ treatment than what has previously been shown for ovariectomized mice.

Physical activity decreases with aging in many species [1]. Similarly in the studies reported here, aged female mice showed relatively low activities. For example, young mice in Study II were active for ~5 hr per day and ambulated about their cages ~1000 m per day while the aged mice in Study I were active for ~4 hr and ambulated only ~400 m per day. Like aging, young female mice that are rendered estrogen-deficient via ovariectomy have 20–55% lower cage activities relative to ovariectomized mice that are replaced with E₂ [7]. Similar to cage activities, wheel running is detrimentally affected by the loss of ovarian hormones via ovariectomy in young mice, and can be preserved with treatment of E₂ [8,9,12,47,49]. These observations led us to test hypothesize that cage activities and wheel running would be enhanced in aged, ovarian-senescent mice in response to E₂ treatment. However, treatment with E₂ evoked a decrease in running, with mice running about half the distances of those that were treated with placebo. It is possible that the dosing regimen used in these mice was not optimal for eliciting wheel running. While there is large range of E₂ doses that induces

rodent wheel running and the dose used in this study was likely close to that which yielded the greatest wheel running in young mice [50]. Another possibility is that perhaps the timing of treatment onset post-ovarian failure was not soon enough to recognize the protect effects of hormone treatment. Clearly, closer analyses of hormone dosing regimens in aged mice are needed.

To explore the possibility that the senescent ovarian tissue influenced wheel running, we compared running between aged mice with intact ovaries to those that had their non-functioning ovaries surgically removed. Interestingly, following the removal of the senescent ovaries, running decreased more than that in age-matched mice that underwent sham surgeries. Although there were low numbers of mice for this study, the result suggests that in the aged mouse, some component of the senescent, follicle-depleted ovary influences wheel running. This would not explain, however, why the outcome of both E₂ treatment and ovariectomy of the aged mice was lowered wheel running (see Figures 2 and 3); these findings also warrant further studies.

We previously reported that in young mice, wheel running as little as 1.45 km per day can induce positive skeletal muscle adaptations [12]. Increases in force due to wheel running have been shown in aged male mice [51]. In Study I reported here, the aged female mice only ran 2.3 km per day and this was enough to produce favorable adaptations in soleus muscle. Irrespective of ovarian hormone status, soleus muscles from mice that wheel ran for 8 weeks had more mass and generated more force compared to mice that did not wheel run. Absolute force and active stiffness were both ~22% greater in mice that ran on wheels. This is an important finding in that muscle adaptations in aged female mice can occur in response to exercise independent of ovarian hormone status.

E₂ treatment in aged mice had a protective effect on soleus muscle fatigue. There has been a noted difference in fatigue resistance between sexes with females exhibiting more fatigue resistance than males [19]. Recently this sex difference was hypothesized to be independent of androgen because androgen receptor knockout mice had equivalent fatigue resistance of the soleus muscle to that of wild type female mice, both of which were more resistant than muscles from wild type males [52]. It is possible that the direct effects of E₂ on soleus muscle fatigue can explain the commonly found sex difference. We have previously determined that E₂-mediated effects on skeletal muscle strength are independent of physical activity, thus we propose that the fatigue results presented in this study are due to direct effects of E₂ on skeletal muscle [7].

Aside from the E₂-mediated improvement in fatigue-resistance, hormone treatment did not affect soleus muscle force generation in aged female mice. A possible explanation is that estrogen receptor content or function could be altered with aging rendering the tissue non-responsive to E₂. Evidence for this idea includes reduced binding of nuclear estrogen receptors in the hypothalamus, pituitary, and uterus of aged, 23-month-old female mice following E₂ treatment [53]. Also, the proportion of nuclei positively stained for estrogen receptor alpha and beta in quadriceps muscle of postmenopausal women were reduced compared to that in muscle of young women [54]. While young ovariectomized mice responded to the loss and treatment of E₂ by changing muscle levels of estrogen receptor alpha [41], it is possible that skeletal muscle of aged mice do not respond as well [55]. Brain areas have been shown to be both E₂-responsive and responsible for physical activity [49,56], thus it is possible that the lack of differences in physical activity of the aged E₂-treated mice could be due to changes in neurologic estrogen receptors. Further detailed studies are needed to determine both content and function of skeletal muscle and nervous system estrogen receptors in aged compared to young females.

The original hypotheses that were put forth and tested in Study II were partially supported. In young mice prematurely ovarian-senescent due to VCD, we found minor effects of E₂ replacement. VCD+E₂ mice spent relatively more time being active in the cage and ambulatory distance tended to be greatest in those mice, but wheel running was not improved. Notably, VCD-induced ovarian senescence did not result in reduced cage activities or wheel running compared to control mice, indicating that the presence of the ovary, even when senescent, may influence physical activity. How the senescent ovary may affect physical activity in mice is speculative. It has been shown that the ovaries of VCD mice produce non-detectable levels of E₂ but still produce low levels of androgen, such that the ratio of androstenedione (the androgen precursor to testosterone) to E₂ in VCD mice is quite high relative to that ratio in control mice with normal estrous cycles [23]. Therefore, it is possible that androgen or the ratio of androgen to E₂ influences wheel running and cage activities. Androgen has been implicated as a behavioral modifier as exemplified by the low running distance and cage activities in androgen receptor knock out mice [57,58]. Thus, interactions of the various sex hormones and their underlying mechanisms by which running motivation in mice is affected requires more research.

Also partially supporting the hypotheses within Study II are the results of the soleus contractile function testing. In young mice with undetectable levels of E₂ due to VCD-induced ovarian senescence, there was no decrement in soleus muscle function as originally hypothesized. However, in support of our hypotheses, VCD+E₂ mice had better soleus muscle strength. Concentric, isometric, and eccentric forces were up to 20% higher in VCD +E₂ mice compared to VCD mice. These results substantiate that in young mice, skeletal muscle is responsive to E₂ treatment.

In summary, these studies report a number of novel findings on physical activity and skeletal muscle in ovarian-senescent mouse models. First, E₂ treatment to aged, ovarian-senescent mice was not affective in increasing physical activities. Ovariectomy of these aged female mice impaired wheel running further, suggesting that some component of the senescent ovary in addition to estrogen production might influence physical activity. Second, E₂ treatment to aged, ovarian-senescent female mice improved muscle fatigue resistance. However, force production was not affected as has been reported previously in young, ovariectomized mice suggesting a diminished responsiveness of skeletal muscle in aged mice. Third, wheel running improved soleus muscle function in aged female mice irrespective of hormone status. Fourth, in young mice exposed to the chemical VCD, producing a senescence specifically of the ovarian tissue, greater soleus muscle force generation occurred following E₂ replacement. These results substantiate the beneficial effect of this hormone on muscle function. Future studies should examine the possibility that the senescent, follicle-depleted ovarian tissue can influence physical activity in female mice and determine whether or not skeletal muscle of aged, ovarian-senescent mice can respond to E₂ treatment.

Highlights

- Wheel running provoked positive adaptations to soleus muscles of aged mice.
- E₂ protected soleus muscle fatigue but not force in aged, ovarian-senescent mice.
- Young, ovarian-senescent mice had greater soleus muscle force with E₂ treatment.

Abbreviations

E ₂	17 β -estradiol
VCD	4-vinylcyclohexene diepoxide

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Figure 1.

Design for Study I; Upon confirmed ovarian-senescence, mice were randomly assigned to sedentary or running groups. Sedentary mice were then randomly assigned to placebo or E₂ treatment, while runners were divided on a rank-order scheme to placebo or E₂ treatment. Design for Study II; Approximately, half of the mice dosed with VCD were randomized to receive E₂ treatment. Physical activity testing (wheel running and cage activities) occurred once prior to and twice following hormone manipulation. In both studies, following ~8 weeks of hormone manipulation, soleus muscle function was tested *in vitro*.

Figure 2.

Distances run on wheels per 24 hr depicted as weekly averages for aged, ovarian-senescent mice treated with placebo or E₂ (means $\pm$ SE). Pre time point was prior to treatment. Data analyzed by 2-way repeated measures ANOVA (treatment by time). Main effect of both treatment and time. N=9–12 per group. *Significantly different than the pre time point; †Significantly different than week 2.

Figure 3. Wheel running distances for aged, ovarian-senescent mice before any surgical procedure and then following a sham operation or ovariectomy (OVX) (means $\pm$ SE). Data analyzed by 2-way repeated measures ANOVA (surgical group by time). *Significantly less than sham at given time. N=3–5 per group.

Figure 4.

Force decrements and recovery from a fatiguing bout of contractions performed by isolated soleus muscles from aged, ovarian-senescent mice following 60 days of ovarian hormone manipulation and being sedentary or wheel running (means $\pm$ SE). Fatigue is calculated as the percentage of force relative to that of the first contraction of the protocol. Plotted are the relative forces of every fifth contraction during the 60-contraction fatiguing protocol. There were main effects of E₂ from 10th – 60th contractions. Insert is pre and post P₀ values for each of the four groups of mice, showing that the fatiguing bout did not induce injury to the soleus muscle because post P₀ values recovered to equivalent levels of pre P₀ values. N=7–12 per group.

Figure 5. Contractility of soleus muscles from mice treated with sesame oil (control), VCD, or VCD +E₂ (means ± SE). Specific forces are corrected for physiological cross-sectional area of soleus muscle. Data analyzed by one-way ANOVA. *Significantly different than VCD; †Significantly different than control. N=5–8 per group.

Table 1

Body and uterine masses from aged, ovarian-senescent mice that were treated with placebo or E₂ for 8 weeks in Study I.

	Sedentary		Runner		2-Way ANOVA P-value		
	Placebo (n=9)	E ₂ (n=10)	Placebo (n=9)	E ₂ (n=12)	Running effect	Estradiol effect	Interaction
Body mass at 20-mo (g)	27.8 ± 1.2	25.6 ± 0.6	27.7 ± 1.6	26.7 ± 0.6	0.602	0.137	0.543
Body mass at 22-mo (g)	28.8 ± 1.3	26.7 ± 0.8	28.7 ± 1.9	26.5 ± 0.5	0.909	0.067	0.917
Uterus mass (mg)	94.4 ± 7.4	199.0 ± 10.3	98.9 ± 10.8	182.5 ± 25.7	0.069	<0.001	0.593

Mean ± SE. Body masses were measured when all mice were ovarian-failed but had not yet been randomized to receive E₂- or placebo-treatment (~20-mo of age) and at the termination of the study (~22-mo of age) after 8 weeks of hormone intervention.

Table 2

In vitro contractile properties of soleus muscles from aged, ovarian-senescent mice treated with placebo or E₂ for 8 weeks in Study I.

	Sedentary		Runner		2-Way ANOVA P-value		
	Placebo (n=9)	E ₂ (n=10)	Placebo (n=9)	E ₂ (n=12)	Running effect	Estradiol effect	Interaction
Soleus mass (mg)	9.4 ± 0.7	8.7 ± 0.5	10.8 ± 0.4	9.6 ± 0.5	0.024	0.076	0.612
Muscle length (mm)	11.3 ± 0.1	11.1 ± 0.2	11.2 ± 0.1	11.2 ± 0.1	0.937	0.292	0.552
P ₁ (mN)	23.6 ± 2.2	24.3 ± 1.7	25.9 ± 1.2	27.9 ± 1.3	0.380	0.899	0.186
Twitch TPT (ms)	40.4 ± 1.5	42.3 ± 1.6	41.9 ± 1.0	44.2 ± 1.5	0.249	0.155	0.890
Twitch RT _{1/2} (ms)	57.6 ± 7.2	52.4 ± 7.7	52.3 ± 4.0	58.1 ± 2.9	0.973	0.949	0.324
P ₀ (mN)	139.5 ± 14.9	133.3 ± 10.5	161.5 ± 9.0	173.3 ± 6.8	0.005	0.788	0.389
Specific P ₀ (N/cm ²)	12.9 ± 1.4	13.0 ± 1.1	12.7 ± 0.7	15.3 ± 0.7	0.314	0.186	0.195
Tetanic +dP/dt (N/s)	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	0.060	0.695	0.547
Tetanic -dP/dt (N/s)	1.9 ± 0.2	1.7 ± 0.3	2.2 ± 0.2	2.2 ± 0.1	0.635	0.519	0.073
Active stiffness (N/m)	272.4 ± 15.2	278.6 ± 23.4	323.9 ± 10.0	341.1 ± 8.0	<0.001	0.716	0.437
Passive stiffness (N/m)	20.9 ± 3.3	17.3 ± 2.8	15.8 ± 1.9	16.3 ± 2.4	0.254	0.567	0.439
Fatigue (% of initial contraction)	49.1 ± 5.7	55.1 ± 6.4	43.3 ± 5.3	63.1 ± 4.0	0.837	0.022	0.206

Mean ± SE. P₁, peak twitch force; TPT, time to peak twitch force; RT_{1/2}, one-half relaxation time; +dP/dt, maximal rate of tetanic force development; -dP/dt, maximal rate of relaxation; P₀, maximal isometric tetanic force; Specific P₀ is normalized by physiological cross sectional area; Fatigue is the percentage of maximal isometric tetanic force on the 60th contraction relative to that of the 1st contraction of the protocol.

Table 3

Physical activities of control mice, mice prematurely ovarian-senescent due to VCD, and VCD mice treated with E₂ in Study II.

	Wheel running (km/24 hr)			2-Way ANOVA		
	Control	VCD	VCD+E ₂	P-value		
	(n=8)	(n=5)	(n=6)	Time effect	Group effect	Interaction
Pre	7.2 ± 0.5	8.0 ± 0.5	8.5 ± 0.3	0.338	0.710	0.434
Week 2	8.0 ± 0.5	8.3 ± 0.6	8.0 ± 0.9			
Week 5	8.5 ± 0.5	8.1 ± 0.5	8.8 ± 0.5			
Total time active in cage (min/24 hr)						
Pre	267 ± 23	288 ± 22	291 ± 21	-	-	0.018
Week 1	270 ± 21	292 ± 33	242 ± 29			
Week 7	270 ± 20	309 ± 5	366 ± 16 ^{*,†,‡}			
Distance ambulated in cage (m/24 hr)						
Pre	756 ± 114	905 ± 170	890 ± 150	0.958	0.064	0.748
Week 1	1389 ± 125	833 ± 169	553 ± 109			
Week 7	1084 ± 379	966 ± 49	1210 ± 115			
Rearing counts (n/24 hr)						
Pre	7203 ± 1245	7144 ± 570	8466 ± 1003	0.044	0.458	0.637
Week 1	8494 ± 1216	9181 ± 1574	8814 ± 1447			
Week 7 [§]	8003 ± 1548	8868 ± 372	11872 ± 740			

Mean ± SE. Pre is the time point prior to hormone treatment. Data were analyzed by 2-way repeated measures ANOVA with time as the repeated factor.

^{*} Significantly different than Pre within group;

[†] Significantly different than Week 1 within group;

[‡] Significantly different than Control within Week 7;

[§] Significantly different than Pre.

Table 4

In vitro contractile properties of soleus muscles from young adult, prematurely ovarian-senescent mice in Study II.

	Control (n=8)	VCD (n=5)	VCD+E ₂ (n=6)	1-way ANOVA P-value
Soleus mass (mg)	9.9 ± 0.4	9.4 ± 0.5	9.9 ± 0.2	0.616
Muscle length (mm)	11.6 ± 0.1	11.7 ± 0.1	11.6 ± 0.2	0.828
P _t (mN)	36.8 ± 1.9	37.5 ± 2.7	38.0 ± 1.4	0.905
Twitch TPT (ms)	38.5 ± 1.0	39.4 ± 1.6	40.6 ± 1.4	0.794
Twitch RT _{1/2} (ms)	43.8 ± 1.3	43.8 ± 1.2	45.3 ± 2.3	0.771
Tetanic +dP/dt (N/s)	2.9 ± 0.1	2.6 ± 0.2	3.4 ± 0.2 *	0.026
Tetanic -dP/dt (N/s)	2.3 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	0.115
Active stiffness (N/m)	291.9 ± 7.4	311.9 ± 9.4	328.6 ± 14.6	0.056
Passive stiffness (N/m)	12.0 ± 0.6	11.4 ± 0.6	11.0 ± 0.2	0.381

Mean ± SE. P_t, peak twitch force; TPT, time to peak twitch force; RT_{1/2}, one-half relaxation time; +dP/dt, maximal rate of tetanic force development; -dP/dt, maximal rate of relaxation.

* Significantly different than VCD.