Appendicular lean mass is lower in late- compared to early- perimenopausal women: potential role of FSH

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Abstract

Age-related declines in skeletal muscle mass (i.e. sarcopenia) contribute to physical disability in older women. Although a menopause-related increase in fat mass is well documented, whether menopause influences muscle mass and sarcopenia is unclear. We determined the extent to which skeletal muscle mass differs across the stages of the menopause transition in women, and whether these differences are associated with estradiol or other sex hormones. This was a cross-sectional study of 144 healthy women (aged 30-70 years), classified as premenopausal (n=30, 38±6yrs; mean ± SD), early (n=31, 50±3yrs) and late perimenopausal (n=30, 50±4yrs), and early (n=26, 55±3yrs) and late postmenopausal (n=27, 62±4yrs). Appendicular lean mass (ALM) adjusted by the square of height in meters (ALM index; ALMi) was assessed using dual-energy x-ray absorptiometry. ALMi was lower (p<0.05) in late perimenopausal and postmenopausal compared to early perimenopausal with no significant differences between other groups (premenopausal, 6.6±0.6; early perimenopausal, 6.8±0.8; late perimenopausal, 6.1±0.8; early postmenopausal, 6.5±1.1; and late postmenopausal, 6.2±0.9 kg/m²). The prevalence of sarcopenia (ALMi ≤5.67 kg/m²) was 7, 3, 30, 27, and 32% in premenopausal, early and late perimenopausal, and early and late postmenopausal group, respectively. ALMi measured across menopause stages was inversely correlated to follicle stimulating hormone (FSH; r=-0.28, p=0.003) but not to estradiol (r=0.088, p=0.34). The menopause transition appears to be a vulnerable period for the loss of skeletal muscle mass that may begin during the late perimenopausal transition. Future studies are necessary to investigate the potential effect of FSH on skeletal muscle.

New & Noteworthy: Our data suggest that the late perimenopausal stage may be a vulnerable period for the loss of skeletal muscle, potentially related to elevations in FSH.
Introduction

Sarcopenia, the age-related decline in skeletal muscle mass and strength, is one of the main contributors to physical disability in older adults (16). Sarcopenia-associated physical disability results in an annual healthcare cost of $18.4 billion in the United States (17). Sarcopenia is not a condition that only affects very old individuals, but can also be observed in middle-aged ambulatory adults (3). Additionally, increasing evidence suggests that there may be a sexual dimorphism in physical disability and sarcopenia. Older women experience greater physical disability and morbidity compared to older men (44) and have a greater prevalence of sarcopenia beginning at the 4th decade and persisting through old age (16). Moreover, a longitudinal study showed that women with severe sarcopenia were more likely than sarcopenic men to develop a physical disability (15). These findings emphasize the need to mitigate the progression of sarcopenia in aging, particularly in older women.

Menopause may contribute to sarcopenia in women. As opposed to andropause which occurs in less than 10% of middle-aged men (10), changes in the sex hormone environment during the menopause transition could be a strong determinant of muscle mass in women. Cross-sectional studies (2, 36, 39) have reported that lean mass is lower in postmenopausal compared to premenopausal women. However, whether muscle mass and the prevalence of sarcopenia differ across the stages of the menopausal transition, specifically during the early- to late-perimenopausal years, is unknown.

The decline in estradiol is believed to be the most important factor in the menopause-related declines in muscle mass. However, estrogen-based hormone therapy (HT) studies that investigated lean body and skeletal muscle mass in postmenopausal women have provided mixed results. Some studies (7, 31, 34) have reported that estrogen-based HT preserves muscle mass in
postmenopausal women, while others (1, 9, 18) have shown no effect. A recent rodent study by Liu et al. (20) raised the possibility that increasing levels of circulating follicle stimulating hormone (FSH) could be another strong factor mediating the decline in skeletal muscle mass during the menopausal transition. Therefore, the purpose of this study was to determine 1) the extent to which appendicular lean mass index (ALMi), a surrogate measure of skeletal muscle mass, differs across the stages of the menopause transition, specifically from the early- to late-perimenopausal years, in healthy women; 2) whether postmenopausal women who previously used HT have a greater ALMi compared to non-users; and 3) whether the reduction in ALMi across menopausal stages is associated with changes in estradiol or other sex hormones (e.g. FSH).

**Materials and Methods**

**Population.** This was a secondary analysis of a cross-sectional study that examined biomarkers of cardiovascular aging across the stages of the menopause transition (25). This secondary analysis includes 144 healthy women aged 30-70 years. Characteristics of the women have been previously described (25). Menopausal status was assessed by self-reported menstrual cycle, and staging was determined according to the Stages of Reproductive Aging Workshop (STRAW) criteria (35). Briefly, premenopausal women (n=30) had regular menstrual cycles (21-35 d), early perimenopausal (n=31) had ≥2 cycles with cycle length changes of ≥7 d, late perimenopausal (n=30) had ≥2 and <12 months of amenorrhea, early postmenopausal (n=26) had ≥1 yr of amenorrhea but ≤5 yr since menopause; and late postmenopausal (n=27) were >5 yr since menopause. Inclusion criteria for this study (25) were: 1) no use of oral contraceptives or HT for at least 6 months (to avoid the effects of estrogen-based HT on vascular function), 2) fasted glucose <126 mg/dL, 3) blood pressure <140/90 mmHg, 4) non-smoker, 5)
sédentaire/recreationally active (<3 days/week vigorous aerobic or resistance exercise; to prevent confounding of the influence of physical activity), and 6) healthy as determined by physical examination, medical history, standard blood chemistries (i.e. normal liver, kidney, and thyroid function) and electrocardiography at rest and during a graded exercise treadmill test. Women with a history of or active estrogen-dependent neoplasms, acute liver or gallbladder disease, cardiovascular disease, venous thromboembolism, hypertriglyceridemia, hysterectomy/oophorectomy, and cancer were excluded. Women had not used anti-inflammatory medications or vitamin supplements for at least 4 weeks so that it would not influence the testing visit. The protocol was approved by the Colorado Multiple Institutional Review Board and participants provided written informed consent.

**Body composition.** Total body lean mass, ALM (the sum of limb lean mass), ALMi (ALM index, ALM adjusted by the square of height in meters), total body fat mass (FM), FMi (FM index, FM adjusted by the square of height in meters) and trunk FM were measured by dual x-ray absorptiometry (DXA, Hologic Discovery, software version 11.2, Hologic Inc, Waltham, MA) as previously described (6, 12-14, 19, 42). The recommendations of the manufacturer were used to define the regions (e.g., arms, legs, trunk). Lines were initially placed by the computer program and then manually adjusted by a technician. The proximal ends of the lines that separated the arms from the trunk were positioned through the middle of the axilla; they were then angled outward from the body so that they separated the arms from the trunk. A pelvic triangle was positioned so that one horizontal line was just superior to the iliac crests and the two vertical lines angled inferiorly to bisect the femoral neck of both hips to between the legs. The coefficient of variation (95% CI) for lean and fat are 0.7% (0.5, 0.8%) and 1.7% (1.3, 2.1%), respectively. Calibration procedures included spine phantom scans daily, whole-body phantom scans three
times per week, air scans once a week, and tissue bar scans once a month. Scans were completed by two trained and experienced technicians and reviewed by an investigator to insure appropriate data acquisition and image analysis.

To confirm the feasibility of DXA (i.e. ALMi) as a surrogate marker of skeletal muscle areas, mid-thigh muscle area was assessed by computed tomography (CT) in a subgroup of women (n=37) as previously described (33, 43). Briefly, axial CT images were obtained 20 cm superior to the distal edge of the lateral condyle of the right femur for measurement of thigh muscle and fat areas (120 kVp, 200-300 maS, and 10 mm slice thickness; General Electric instrument; Waukesha, WI) by experienced technicians in the University of Colorado Hospital Department of Radiology. Images were analyzed by the technicians at the CT Scan Reading Center. Thigh muscle area was separated from subcutaneous fat area by manually tracing along the deep fascial plane surrounding the muscles. Thigh muscle areas were averaged over the right and left thigh slices. Threshold for inclusion of repeat thigh scans was ±1 cm of baseline scan location.

Analysis programs were developed by the University of Colorado CT Reading Center using IDL software (RSI, Inc., Boulder, CO) on a Sparc 20 workstation (Sun Microsystems, Sunnyvale, CA). Scans in the current study were completed by two trained and experienced technicians and reviewed by an investigator to insure appropriate data acquisition and image analysis. Minimal waist circumferences were measured according to published guidelines (21).

**Definition of sarcopenia.** A sex-specific ALMi cut point of ≤5.67 kg/m² was used to define sarcopenia and determine the prevalence of sarcopenia in each menopause stage, in accordance with the International Working Group on Sarcopenia (IWGS) (3) and Newman et al. (27). Secondarily, we determined the study-specific prevalence of sarcopenia in each menopause stage.
using the ALMi cut point of $\leq 5.31 \text{ kg/m}^2$, corresponding to $\geq 2$ standard deviations (SD) below the mean ALMi of the premenopausal women in the present study.

**Sex hormones.** Serum levels of estradiol, FSH, and progesterone were measured using chemiluminescence, total testosterone using a one-step competitive assay, and estrone using radioimmunoassay by the Colorado Clinical and Translational Sciences Institute (CCTSI) Clinical and Translational Research Center (CTRC) core laboratory, as previously described (25). The coefficient of variation (95% CI) for each hormone are as follows: intra-assay CV: estradiol, 4.3%; estrone, 11.5%; FSH, 1.8%; progesterone, 4.4%; testosterone, 2.1%; and inter-assay CV: estradiol, 8.2%; estrone, 19.8%; FSH, 3.8%; progesterone, 7.9%; and testosterone, 5.1% (24).

**Physical activity, aerobic exercise capacity, and energy intake.** Physical activity level was determined objectively by pedometer step counts (23), and leisure time physical activity (LTPA) determined subjectively using the Modifiable Activity Questionnaire (28). This questionnaire included the frequency of participation in different activities (work, heavy and light exercise, etc), the number of hours spent in sedentary activities (watching TV, sitting, etc), and finally calculated those in total metabolic equivalent task (MET) per week. Peak oxygen consumption (i.e. the maximal aerobic exercise capacity) was determined using an incremental treadmill running protocol as described previously (40). Energy intake and dietary macronutrient composition (i.e. carbohydrate, fat, and protein in grams) were determined from 3-day food intake records as described previously (37). The CCTSI CTRC Nutrition Core analyzed the dietary food records (41).

**Statistical analysis.** All data elements were examined using descriptive statistics and graphical summaries (boxplots, profile plots); skewed distributions were improved by transformation.
Results are presented as mean ± SD for normally distributed variables, or median and the interquartile range for skewed descriptive variables (i.e., estradiol, estrone, progesterone, testosterone, and LTPA). One-way analysis of variance (ANOVA) was used to determine the main effects of menopause stage on participant characteristics, sex hormones, and body composition. Tukey HSD post hoc tests were used to identify differences among menopause stages. Because prior hormone therapy use could influence the ALM, two-way ANOVA was used to determine the menopause stage main effect (early vs. late postmenopausal), condition main effect (HT-users vs. -nonusers), and stage x condition interaction for ALMi and FMi. Exploratory analyses were conducted using Bivariate Pearson’s correlations to test the association between ALMi and variables of interest (e.g., sex hormones, physical activity markers, CT data). If there was a significant correlation found, partial correlations were used to assess whether the significance is maintained after adjusting for other variables (e.g. age, estradiol, estrone). All data were analyzed using IBM SPSS Statistics version 24.0 (IBM/SPSS, Armonk, NY). P<0.05 was considered statistically significant.

Results

Participants. There was a main effect of menopause stage on age, total lean mass, ALM, trunk FM, concentrations of estradiol, estrone, FSH, and progesterone and peak aerobic capacity (Table 1, all p<0.05). Thirty-one percent and 48% of early and late postmenopausal women, respectively, were prior HT users (average duration: 2.9±2.8 and 4.8±3.6 yrs, respectively).

ALMi and FMi. Compared to early perimenopausal, ALMi was lower in late perimenopausal and late postmenopausal (p=0.011 and 0.048, respectively) with no significant differences between other menopause stages (Figure 1A). Although there was a trend for FMi to be elevated across menopause stages (p=0.06, premenopausal vs. early postmenopausal), FMi was not
significantly different between early and late perimenopausal women (Figure 1B). ALMi was
lower in HT-users than nonusers in both early and late postmenopausal stages (main effect of HT
use, p=0.04, Figure 2A). There were no significant differences in FMi between HT-users and
nonusers (Figure 2B).

Prevalence of sarcopenia. When the ALMi cut point of 5.67 kg/m² was used (3, 27), the
prevalence of sarcopenia was 7, 3, 30, 27, and 32% in premenopausal, early and late
perimenopausal, and early and late postmenopausal, respectively. The study-specific prevalence
of sarcopenia (ALMi ≤ 5.31 kg/m²; ≥2SD below premenopausal) was 0, 0, 10, 11.5, and 18.5%
in premenopausal, early and late perimenopausal, and early and late postmenopausal,
respectively (Table 2).

Dietary intake and physical activity. We found no significant main effects of menopause stage
on energy intake, carbohydrate, fat, or protein consumption (Table 3). The results were
unchanged after adjusting for lean mass and total body mass (data not shown). There was no
significant main effect of menopause stage on physical activity markers (i.e. pedometer or LTPA,
Table 3).

Associations. In the pooled population, ALMi was not correlated with estradiol concentrations
(r=0.09, p=0.50) but was negatively correlated with FSH (r=-0.28, p=0.003), and positively
correlated with protein intake (r=0.25, p=0.02) and FMi (r=0.50, p<0.001, Table 4). Within
premenopausal, ALMi was negatively correlated with estradiol (r=-0.44, p=0.04), whereas
within perimenopausal women (early and late combined), FSH was negatively correlated with
ALMi (r=-0.28, p=0.046). ALMi was positively correlated with FMi in premenopausal (r=0.55,
p=0.002), perimenopausal (r=0.42, p=0.001), and postmenopausal (r=0.65, p<0.001) women.
The negative correlations between ALMi and FSH in the pooled population, and within the peri- and post-menopausal groups were mostly maintained or tended to be significant after adjusting for age, estradiol, estrone, progesterone, testosterone, dietary intake, protein intake, LTPA, pedometer, and FMi (Table 4, Figure 3). ALMi was strongly correlated with mid-thigh muscle area (CT analysis; $r=0.66, p<0.0001$).

Discussion

The present study provides novel insights into potential factors that may contribute to sarcopenia in older women. To our knowledge, we are the first to demonstrate that the menopause transition, particularly the late perimenopausal stage, may be a vulnerable period for the loss of skeletal muscle mass, as indicated by a lower ALMi in late- compared to early- perimenopausal women. We found that the reduction in ALMi across menopausal stages was not associated with lower estradiol, but rather with higher FSH levels. Moreover, early and late postmenopausal women who had previously used estrogen-based HT had a lower ALMi than women who never used estrogen-based HT.

Menopause and muscle mass. It is becoming increasingly evident that menopause may impact muscle mass in women. Cross-sectional studies (2, 36, 39) have reported lower lean mass in post- compared to pre-menopausal women. However, ALMi across the menopausal transition has not been well characterized. The present study provides new information on ALMi across the stages of the menopause transition in healthy women. Compared to early perimenopausal women, ALMi was significantly lower in late perimenopausal and late postmenopausal women with no significant differences among the other menopausal stages. It was surprising that ALMi was not significantly different in early postmenopausal compared to early perimenopausal women, however the greater variability in ALMi in this group suggests diverse responses in muscle mass.
during the early postmenopausal years. This variability may be partially attributed to the fact that a third of the early postmenopausal women were prior HT users, and thus had not experienced prolonged estrogen deficiency at the time of the study. To our knowledge, no cross-sectional study has demonstrated a significant decline in ALMi from early- to late-perimenopause. Human experimental studies (30, 45) using gonadotropin-releasing hormone agonist (GnRH$_{AG}$) treatments partially support our findings in that chronic (4-6 months) suppression of ovarian hormones caused a decrease in total body lean mass in eugonadal women. Our group has also reported a decrease in total body lean mass in healthy premenopausal women with 5 months of GnRH$_{AG}$ treatment (22). Because age and menopause could not be uncoupled in the present study, future investigations using an intervention approach (e.g. GnRH$_{AG}$ treatment) are necessary to isolate the effects of the loss of ovarian hormones from the effects of aging on ALM, and the underlying mechanism(s) (e.g., skeletal muscle protein metabolism).

**Menopause and prevalence of sarcopenia.** Sarcopenia has become one of the most important geriatric conditions and a key risk factor to the development of disability and frailty (16, 26). In a longitudinal comparison of 4504 older adults, sarcopenic women were more likely than men to develop a physical disability (15), suggesting a critical role of sarcopenia in women’s health and quality of life. In the present study, the prevalence of sarcopenia (ALMi ≤5.67 kg/m$^2$) was 3 fold higher in late perimenopausal and postmenopausal compared to premenopausal and early perimenopausal women. In most of previous cross-sectional studies, the prevalence of sarcopenia was studied in women > 65 years of age. Using an ALMi assessed by DXA in the Health Aging and Body Composition (Health ABC) study, Newman et al. (27) reported that the prevalence of sarcopenia (i.e. ALMi ≤5.67 kg/m$^2$) was ~20% in older postmenopausal women (n=1549; aged 70-79 years). Furthermore, Iannuzzi-Sucich et al. (11) found the prevalence of sarcopenia (i.e.
ALMi ≤5.45 kg/m²; ≥2SD below the reference group (4)) was ~23% in women (n=195; aged 75±5 years). The prevalence of sarcopenia reported in these studies was lower than that of the postmenopausal women in the present study (i.e. ~33%). When we used the study-specific sarcopenia criterion of ALMi ≤5.31 kg/m² (≥2SD below premenopausal), the prevalence of sarcopenia was reduced in each stage, and the prevalence of sarcopenia in the late postmenopausal group was comparable to previous studies (11, 27), suggesting that study-specific reference groups are essential to understanding the scope of sarcopenia. An interesting finding in the present study was the increasing prevalence of sarcopenia and decreased ALMi from early- to late-perimenopause, regardless of the sarcopenia criterion. This suggests that perimenopause is a vulnerable period for skeletal muscle mass.

**Estradiol, FSH, and skeletal muscle mass.** Although estrogen deficiency is believed to be the most important factor contributing to loss of lean body mass with menopause, the evidence is mixed. Estrogen-based HT has been shown to preserve skeletal muscle and lean body mass in postmenopausal women (7, 34). In monozygotic postmenopausal twin dyads (31), women treated with estradiol-based HT for 7 years had greater thigh muscle mass, muscle power, and mobility compared to their non HT-using twin. Whole body fat-free mass was reduced in premenopausal women treated with 5 months of GnRH<sub>AG</sub> (i.e. suppression of overall ovarian hormones), but not in those treated with GnRH<sub>AG</sub> plus estradiol add-back (22). Contrary to these findings, the present study found lower ALMi in HT users compared to non-users in both early- and late-postmenopausal women. Moreover, there was no correlation between estradiol concentrations and ALMi in the pooled population and ALMi was inversely correlated with estradiol only in the pre-menopausal women. Taken together, these findings clearly indicate the need for mechanistic...
studies to test whether estradiol is a catabolic or anti-catabolic agent in muscle mass at different stages of the menopause transition.

FSH has recently been implicated as a potential mediator of metabolic actions traditionally attributed to the loss of estradiol. Using an antibody targeting the subunit of FSH, previous studies (38, 46) have suggested that blocking FSH increases bone mass in rodents. A recent paper by Liu et al. (20) expanded these findings to adipose tissues. They demonstrated that rodents treated with the FSH antibody had sharply reduced adiposity, increased brown adipose tissue, beiging of white adipocytes (e.g., enhanced UCP1 expression and mitochondrial density), and increased resting energy expenditure. These findings raise the possibility that FSH also plays a critical role in skeletal muscle mass, one of the most metabolically active organs. To our knowledge, no human or clinical studies have reported whether the rise in FSH during the perimenopausal years is associated with the decline in skeletal muscle mass with aging in women. In a study conducted in healthy postmenopausal women (aged 48-65 years), Garcia-Martin et al. (5) reported an inverse correlation between ALMi and FSH. Similar to the present study, they found no association between ALMi and estradiol concentrations. Our group (22, 33) has previously shown that ovarian suppression of both estradiol and FSH with GnRH$_{AG}$ treatment increased abdominal adiposity and decreased resting energy expenditure and bone mineral density in healthy premenopausal women. Thus, it is possible that beneficial effects of decreased FSH were counteracted, in part, by the unfavorable effects of decreased estradiol. The present study could stimulate paradigm-shifting research investigating the actions of other sex hormones, specifically FSH, on skeletal muscle mass and function in women.

**Experimental considerations and potential limitations.** The aim of the study was to examine ALM across stages of the menopause transition, and specifically examine the association with
estradiol and FSH concentrations. It is recognized that the menopause transition is associated
with changes in metabolic, inflammatory and other factors that could influence ALM. However,
it was beyond the scope of this secondary analyses to examine these or other parameters in the
present study. The present study also has other important limitations that need to be considered.
The first important limitation is the lack of physical function (e.g., muscle strength, mobility)
measures to define sarcopenia, as suggested by the International Working Group on Sarcopenia
(3). Although sarcopenia is most often associated with advanced age and mobility impairments
(3), the present study provides provocative evidence for changes in muscle mass early in the
menopause transition in women who are younger, generally healthy, and sedentary or
recreationally active. Second, the present study was a secondary analysis of body composition
data from an investigation of the biological mechanisms underlying cardiovascular dysfunction
with aging and estrogen deficiency in healthy women (25). As such the present study was not
powered to detect differences in ALMi and to make correlations between ALMi and other
variables, and the results should be interpreted cautiously. Third, the cross-sectional design
precludes discussion of causality. Because menopause stage was determined by self-reported
menstrual cycle characteristics, we cannot rule out the possibility that participants have been
misclassified. However, circulating sex hormone levels for each of the menopausal stages were
comparable to values reported by others (8). Fourth, the interpretation of a single cross-sectional
measurement in ALMi is potentially limiting. Future longitudinal studies with a longer history of
individual changes in body composition and sex hormones are needed. Fifth, as mentioned,
strength is an important component for defining sarcopenia, and we do not have a measure of
strength. Previous studies (29, 32) partially support our finding in that muscle strength also
declines around the time of menopause. Whether the late perimenopausal transition would be a
vulnerable period for the loss of both skeletal muscle strength and mass should be investigated in future studies. Sixth, this study found that ALMi positively correlated with protein intake. This study focused more on the hormonal factors potentially contributing to muscle mass during the peri-menopausal transition, thus, we did not discuss in depth dietary protein intake. This is an important topic that should be studied in the future. Additionally, although we requested that all participants attend their study visits well-hydrated, we did not measure hydration status, per se, and thus, it is possible that hydration status influenced the DXA measure. Lastly, DXA measures bone-free lean tissue mass in the limbs (ALMi) as a surrogate marker for muscle mass. However, our additional CT analysis in a subgroup of participants showed that ALMi by DXA was strongly correlated with mid-thigh muscle area ($r=0.66$, $p<0.0001$).

**Conclusions.** The menopausal transition appears to be a vulnerable period for the loss of ALMi that may begin during the late perimenopausal stage. The loss of ALMi was not associated with declines in estradiol, but rather with elevated FSH levels. These data suggest that the menopausal transition may trigger mechanisms underlying sarcopenia in women. Thus, the perimenopausal years may be a critical time to introduce strategies that mitigate the changes in muscle mass that contribute to physical disability and frailty later in life. Potential mechanisms underlying menopause-related loss of muscle mass, including the role of FSH, should be explored in the future.

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Disclosures

All authors (YP, CJ, CO, KH, WK, and KM) have no conflicts of interest to disclose.

Ethical Approval

Written informed consent was obtained for all participants. The study was approved by the Colorado Multiple Institutional Review Board.
References


Figure Legends

Figure 1. (A) Appendicular lean mass index (ALMi) and (B) fat mass index (FMi). Values are means ± SD. ALMi and FMi = ALM and FM adjusted by height (m²). Pre=premenopausal; EPeri=early perimenopausal; LPeri=late perimenopausal; EPost=early postmenopausal; LPost=late postmenopausal. * p<0.05 versus EPeri; ‡ p=0.06 versus Pre.

Figure 2. Comparisons of estrogen-based hormone therapy (HT) users and non-users on (A) appendicular lean mass index (ALMi) and (B) fat mass index (FMi). Values are means ± SD. ALMi and FMi = ALM and FM adjusted by height (m²). EPost=early postmenopausal; LPost=late postmenopausal. Stage = main stage effect; Condition = main condition effect; S x C = stage x condition interaction; ND= no stage (EPost vs LPost) and condition (non-HT vs HT) difference.

Figure 3. Association. Follicle stimulating hormone (FSH) versus appendicular lean mass index (ALMi, ALM adjusted by height). 144 healthy women (aged 30-70 years). *Denotes a significant correlation.
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<th>LPeri n=30</th>
<th>EPost n=26</th>
<th>LPost n=27</th>
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<td>165±6</td>
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<tr>
<td>Estradiol, pg/mL a,b</td>
<td>79[64, 110]</td>
<td>70[37, 141]</td>
<td>34[10, 94]</td>
<td>11[10, 15]</td>
<td>10[10, 14]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estrone, ng/dL a,b</td>
<td>61[41, 70]</td>
<td>60[34, 88]</td>
<td>43[30, 69]</td>
<td>26[24, 33]</td>
<td>26[23, 37]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH, μIU/mL b</td>
<td>6.5±3.4</td>
<td>22.0±30.0</td>
<td>64.1±35.5</td>
<td>72.1±26.1</td>
<td>84.1±33.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone, ng/dL a,b</td>
<td>0.4[0.2, 0.6]</td>
<td>0.5[0.2, 0.8]</td>
<td>0.3[0.2, 0.5]</td>
<td>0.3[0.1, 0.4]</td>
<td>0.2[0.1, 0.4]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Testosterone, ng/dL a,b</td>
<td>24[22, 33]</td>
<td>22[17, 35]</td>
<td>20[17, 25]</td>
<td>18[17, 23]</td>
<td>17[17, 35]</td>
<td>0.32</td>
</tr>
<tr>
<td>VO₂peak, mL/kg/min c</td>
<td>31.2±6.4</td>
<td>28.3±4.8</td>
<td>27.5±5.9</td>
<td>26.3±3.6</td>
<td>24.7±7.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Note.* Data are mean±standard deviation or a median [interquartile range]. Pre=premenopausal; EPeri=early perimenopausal; LPeri=late perimenopausal; EPost=early postmenopausal; LPost=late postmenopausal; BMI=body mass index; WC=waist circumference; ALM=appendicular lean mass; FSH=follicle stimulating hormone; VO₂ peak=peak aerobic capacity; b n= 118, c n= 139.
Table 2. Prevalence of sarcopenia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre (n=30)</th>
<th>EPeri (n=31)</th>
<th>LPeri (n=30)</th>
<th>EPost (n=26)</th>
<th>LPost (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 5.31 kg/m² of ALMi&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopenic, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (10.0)</td>
<td>5 (11.5)</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>Below 5.67 kg/m² of ALMi&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopenic, n (%)</td>
<td>2 (6.7)</td>
<td>1 (3.2)</td>
<td>9 (30.0)</td>
<td>7 (26.9)</td>
<td>9 (33.3)</td>
</tr>
</tbody>
</table>

Note. Pre=premenopausal; EPeri=early perimenopausal; LPeri=late perimenopausal; EPost=early postmenopausal; LPost=late postmenopausal; ALMi=appendicular lean mass adjusted by height (m²); <sup>a</sup>a cut-point from Fielding et al. [3] and Newman et al. [22]; <sup>b</sup> ≥2SD below the premenopausal group of the current study.
Table 3. Dietary intake and physical activity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>EPeri</th>
<th>LPPeri</th>
<th>EPost</th>
<th>LPost</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>1598±697</td>
<td>1943±565</td>
<td>1791±452</td>
<td>1864±434</td>
<td>1724±478</td>
<td>0.445</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>185±84</td>
<td>238±89</td>
<td>217±78</td>
<td>222±76</td>
<td>198±75</td>
<td>0.416</td>
</tr>
<tr>
<td>Fat, g</td>
<td>66±35</td>
<td>72±25</td>
<td>70±21</td>
<td>73±27</td>
<td>74±23</td>
<td>0.937</td>
</tr>
<tr>
<td>Protein, g</td>
<td>71±29</td>
<td>85±23</td>
<td>77±25</td>
<td>76±23</td>
<td>71±19</td>
<td>0.457</td>
</tr>
<tr>
<td><strong>Physical activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedometer, steps/day</td>
<td>5927±2314</td>
<td>7364±2491</td>
<td>6380±2067</td>
<td>7231±3535</td>
<td>7648±2761</td>
<td>0.267</td>
</tr>
</tbody>
</table>

Note. Data are mean±standard deviation or a median [interquartile range]. Pre=premenopausal; EPeri=early perimenopausal; LPPeri=late perimenopausal; EPost=early postmenopausal; LPost=late postmenopausal; LTPA=leisure time physical activity; MET metabolic equivalent; n of dietary intake = 85; n of pedometer = 98; n of LTPA = 117.
Table 4. Correlates of appendicular lean mass index (ALMi) by menopausal categories.

<table>
<thead>
<tr>
<th></th>
<th>ALMi Pooled</th>
<th>ALMi Pre</th>
<th>ALMi Peri</th>
<th>ALMi Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>-0.128</td>
<td>0.148</td>
<td>-0.114</td>
<td>-0.169</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.088</td>
<td>-0.441*</td>
<td>0.094</td>
<td>0.077</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.115</td>
<td>-0.261</td>
<td>0.037</td>
<td>0.283†</td>
</tr>
<tr>
<td>FSH</td>
<td>-0.275**</td>
<td>-0.011</td>
<td>-0.281*</td>
<td>-0.289†</td>
</tr>
<tr>
<td>Progesterone</td>
<td>-0.025</td>
<td>0.164</td>
<td>-0.054</td>
<td>-0.180</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.105</td>
<td>0.050</td>
<td>0.079</td>
<td>0.121</td>
</tr>
<tr>
<td>Dietary intake</td>
<td>0.205†</td>
<td>0.471</td>
<td>0.239</td>
<td>0.180</td>
</tr>
<tr>
<td>Protein intake</td>
<td>0.251*</td>
<td>0.306</td>
<td>0.277†</td>
<td>0.276</td>
</tr>
<tr>
<td>LTPA</td>
<td>-0.049</td>
<td>-0.192</td>
<td>0.035</td>
<td>-0.065</td>
</tr>
<tr>
<td>Pedometer</td>
<td>-0.133</td>
<td>-0.090</td>
<td>-0.089</td>
<td>-0.136</td>
</tr>
<tr>
<td>FMi</td>
<td>0.501***</td>
<td>0.547**</td>
<td>0.423**</td>
<td>0.651***</td>
</tr>
</tbody>
</table>

Partial Correlations of ALMi with FSH

<table>
<thead>
<tr>
<th></th>
<th>ALMi Pooled</th>
<th>ALMi Pre</th>
<th>ALMi Peri</th>
<th>ALMi Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>-0.215*</td>
<td>-0.037</td>
<td>-0.272†</td>
<td>-0.270†</td>
</tr>
<tr>
<td>Estradiol</td>
<td>-0.281**</td>
<td>0.003</td>
<td>-0.267†</td>
<td>-0.281†</td>
</tr>
<tr>
<td>Estrone</td>
<td>-0.251**</td>
<td>-0.076</td>
<td>-0.280*</td>
<td>-0.242</td>
</tr>
<tr>
<td>Progesterone</td>
<td>-0.286**</td>
<td>0.036</td>
<td>-0.298*</td>
<td>-0.262†</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-0.267**</td>
<td>0.010</td>
<td>-0.278†</td>
<td>-0.290†</td>
</tr>
<tr>
<td>Dietary intake</td>
<td>-0.297**</td>
<td>-0.401</td>
<td>-0.322*</td>
<td>-0.282</td>
</tr>
<tr>
<td>Protein intake</td>
<td>-0.282**</td>
<td>-0.452</td>
<td>-0.313†</td>
<td>-0.261</td>
</tr>
<tr>
<td>LTPA</td>
<td>-0.272**</td>
<td>-0.019</td>
<td>-0.274†</td>
<td>-0.330*</td>
</tr>
<tr>
<td>Pedometer</td>
<td>-0.254*</td>
<td>0.209</td>
<td>-0.300†</td>
<td>-0.280†</td>
</tr>
<tr>
<td>FMi</td>
<td>-0.366***</td>
<td>-0.018</td>
<td>-0.284*</td>
<td>-0.234</td>
</tr>
</tbody>
</table>

Note. ALMi = appendicular lean mass normalized to height in meters²; FSH = follicle stimulating hormone; LTPA = leisure time physical activity; FMi =fat mass normalized to height in meters²; Pre = premenopausal; Peri = early and late perimenopausal; Post = early and late postmenopausal women including both hormone therapy users and non-users; Pooled= Pre + Peri + Post; †p≤0.10; *p<0.05; **p≤0.01; ***p≤0.001.
Figure 1

A

ALMi (kg/m²)

B

FMi (kg/m²)

Pre  EPeri  LPeri  EPost  LPost
Menopausal Stage

Pre  EPeri  LPeri  EPost  LPost
Menopausal Stage

*  *  ‡
Figure 2

A

Stage, $p=0.563$
Condition, $p=0.04$
$S \times C$, $p=0.703$

B

ND
Figure 3

\[ r = -0.275, p = 0.003 \]