Adaptations with Intermittent Exercise Training in Post- and Premenopausal Women

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ABSTRACT

SEIDELIN, K., M. NYBERG, P. PIIL, N. R. JØRGENSEN, Y. HELLSTEN, and J. BANGSBO. Adaptations with Intermittent Exercise Training in Post- and Premenopausal Women. *Med. Sci. Sports Exerc.*, Vol. 49, No. 1, pp. 96–105, 2017. **Introduction**: The purposes of the present study was to examine the effect of intermittent exercise training on musculoskeletal and metabolic health in postmenopausal (PM) and premenopausal (PRM) women and, furthermore, to evaluate whether the adaptations can be maintained with a reduced training frequency. **Methods**: Eighteen PM (51 ± 1 yr, mean ± SEM) and 12 PRM (48 ± 1 yr) women participated in floorball training approximately two times per week for 12 wk. In a subgroup (n = 9) of PM women (PM40), exercise training was performed for an additional 40 wk with a reduced training frequency of approximately one training session per week. **Results**: In PM, the body fat percentage decreased ($P < 0.05$) and the total lean leg mass increased ($P < 0.05$) during the 12-wk training period, with no changes in PRM. In both PM and PRM, lean body mass and maximal oxygen uptake ($\dot{V}O_{2max}$) were higher, and Yo-Yo intermittent endurance test 1 (YYIET-1) performance was better ($P < 0.05$) after the 12-wk training period. Procollagen type 1 amino-terminal propeptide was higher ($P < 0.05$) in PM, and total leg bone mineral density (BMD) was higher ($P < 0.05$) in both PM and PRM after the 12-wk training period. In PM40, total lean leg mass, $\dot{V}O_{2max}$, YYIET-1 performance, level of procollagen type 1 amino-terminal propeptide, and total leg BMD were maintained, whereas whole-body BMD and glycated hemoglobin (HbA1c) were reduced ($P < 0.05$) and the expression of muscle glucose transporter type 4 was higher ($P < 0.05$). **Conclusion**: Twelve weeks of intermittent exercise training increased BMD, intermittent exercise capacity, and $\dot{V}O_{2max}$ in PM and PRM, with PM also having positive changes in body composition. Additional 40 wk of training with a reduced frequency was sufficient to preserve these physiological adaptations and also improve blood glucose regulation in PM. **Key Words:** BONE FORMATION MARKER, BMD, HbA1c, GLUT-4, BODY COMPOSITION, FLOORBALL, MENOPAUSE

The postmenopausal (PM) transition is associated with an increased risk of metabolic syndrome (29) along with an accelerated loss of muscle mass and muscle strength (22), and the most rapid bone loss in women is seen in the menopausal transition (11). However, to what extent these adverse changes are a consequence of the menopausal transition and/or aging remains unresolved as most studies have been conducted on women separated by more than 15 yr. By investigating women of similar age (<5 yr), but with different menopausal status, it is possible to differentiate between these intimately linked processes, as significant age-related physiological changes are likely to be limited within this short time frame.

PM status is associated with a 60% increased risk of metabolic syndrome (29), which leads to diabetes and cardiovascular disease (8). The metabolic syndrome is associated with increased blood levels of glycated hemoglobin (HbA1c) because of impaired blood glucose regulation (31) and increases in body fat as well as decreases in muscle mass (8). The lowering of muscle mass and strength in PM women is associated with loss of size and number of fast twitch type II muscle fibers (20), but whether this is primarily caused by aging or the menopausal transition is unclear.

Estrogen deficiency associated with menopause impairs bone remodeling by increasing bone resorption without an equivalent change in the formation, causing an overall increased bone loss (33). The most widely used marker for development of osteoporosis is bone mineral density (BMD), and because osteoporotic fractures are a significant cause of morbidity and mortality (16), BMD improvement in PM women is of particular interest. In addition to BMD, circulating bone resorption marker carboxy terminal type 1 collagen cross-links (CTX-1), bone formation marker procollagen type 1 amino-terminal propeptide (P1NP), and bone turnover marker osteocalcin (OC), called bone turnover markers, are useful to show changes in bone formation status before changes in BMD are detectable (33).

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Intermittent exercise training has been suggested to be more beneficial than continuous moderate exercise training in reducing the risk of developing metabolic syndrome (34) and for improving body composition in women (35). Adaptations in BMD vary with different types of exercise, but intermittent high-intensity sports such as football, basketball, and racket games that expose the bones to various muscle and ground reaction forces have been suggested to have the highest potential in preserving BMD in PM women (23). Floorball, which is a team sport, resembling hockey, but played indoor with plastic sticks, is characterized by intermittent exercise with multidirectional muscle and ground reaction forces. Hence, floorball training would be expected to be a potent stimulus to improve musculoskeletal and metabolic health in premenopausal (PRM) and PM women. Importantly, the osteogenic responsiveness to mechanical load is reduced in PM compared with PRM women (4). This altered responsiveness may be in part related to estrogen deficiency as PM women on estrogen treatment display enhanced training-induced bone adaptations compared with nontreated PM women (38). Likewise, it has been suggested that exercise training combined with estrogen treatment of recent PM women may be beneficial for preserving or improving muscle function (32). However, more studies are needed to confirm these important roles for estrogen. One approach to study the effects of high levels of estrogen and associated hormones on training adaptations is by studying PRM and PM women of similar age as this eliminates any detrimental effects of age. This has been a confounding factor in the majority of the previous studies as these have included PRM and PM women that were separated by more than 15 yr. Thus, interventions that can improve musculoskeletal and metabolic health in women, irrespective of estrogen levels, are of great clinical relevance given the concerns related to the potential harmful effects of estrogen supplementation in PM women (10).

The aim of the present study was twofold: 1) to determine whether recent PM women obtain similar changes as PRM women of similar age in body composition, physical performance, BMD, bone markers, and blood glucose with 12 wk of floorball training twice a week and 2) to examine if these parameters can be improved or preserved in recent PM women with additional 40 wk of floorball training only one time a week. In particular, CTX-1, P1NP, OC, total leg BMD, and total lean leg mass were used as a primary outcome for the assessment of musculoskeletal health, whereas HbA1c and fasting glucose as well as insulin were used to assess metabolic health.

We hypothesized that floorball training would lead to greater improvements in musculoskeletal and metabolic health in PRM women compared with PM women. Furthermore, that musculoskeletal and metabolic improvements from 12 wk of floorball training could be maintained with only one training session per week for additional 40 wk.

METHODS

Subjects. Eighteen PM women (age = 52 ± 1 yr, mean ± SEM) with maximal oxygen uptake ($\dot{V}O_{2max}$) of 33.3 ± 1.3 mL·min^{-1}·kg^{-1} and 12 PRM (age: 48 ± 1 yr) women with $\dot{V}O_{2max}$ of 34.6 ± 2.1 mL·min^{-1}·kg^{-1} women participated in the study (Table 1). None of the subjects were on hormone replacement therapy or were taking oral contraceptives. All PRM subjects were menstruating regularly, whereas subjects in PM had not experienced a menstrual cycle during the previous 12 months but were less than 3 yr past their final menstrual period. Menopausal status was verified by measurements

TABLE 1. Age, body composition, and sex hormone levels for PM (n = 18) and PRM (PRM, n = 12) women before and after a 12-wk period with floorball training.

	PM (n = 18)		PRM (n = 12)	
	Before	After	Before	After
Age (yr)	52 ± 1****		48 ± 1	
Height (m)	1.68 ± 0.02		1.71 ± 0.02	
Weight (kg)	70.5 ± 3.0	70.6 ± 3.1 (0.1%)	67.0 ± 4.0	67.8 ± 4.2 (1.1%)
Body mass index (kg·m^{-2})	24.8 ± 0.8	24.8 ± 0.8 (0.1%)	22.8 ± 0.9	23.1 ± 1.0 (1.1%)
Body fat (%)	38.6 ± 1.7****	36.4 ± 1.9 (2.2%)***	31.5 ± 2.3	31.1 ± 2.3 (0.5%)
Lean body mass (kg)	41.1 ± 1.2	42.6 ± 1.3 (3.6%)***	43.3 ± 1.5	44.0 ± 1.5 (1.6%)*
Total lean leg mass (kg)	14.7 ± 0.5	15.4 ± 0.7 (4.6%)*	15.9 ± 0.6	16.4 ± 0.6 (3.3%)
Whole-body BMD (g·cm^{-2})	1.19 ± 0.02	1.20 ± 0.02 (0.3%)	1.18 ± 0.02	1.19 ± 0.02 (0.8%)
$\dot{V}O_{2max}$ (mL O_2·min^{-1})[a]	2311 ± 97	2411 ± 101 (4.4%)**	2222 ± 134	2324 ± 124 (5.1%)*
YYIET-1 (m)[b]	713 ± 89	1000 ± 120 (42.5%)***	1098 ± 234	1404 ± 223 (40.6%)***
CM jumping height (cm)[b]	25.1 ± 0.9****	26.6 ± 1.1 (3.7%)*****	30.8 ± 1.4	31.8 ± 1.3 (7.6%)
Estradiol (nmol·L^{-1})[c]	0.10 ± 0.03****		0.43 ± 0.15	
Progesterone (nmol·L^{-1})[c]	1.07 ± 0.16		1.24 ± 0.18	
FSH	71.6 ± 6.7******		13.5 ± 2.8	
LH (IU·L^{-1})[c]	37.0 ± 3.2******		8.6 ± 1.6	
Testosterone[c]	0.80 ± 0.14		0.73 ± 0.11	

Values are expressed as means ± SEM. CM, countermovement; FSH, follicle-stimulating hormone; LH, leutinizing hormone.
*Significantly different from before training, $P < 0.05$.
**Significantly different from before training, $P < 0.01$.
***Significantly different from before training, $P < 0.001$.
****Significantly different from PRM, $P < 0.05$.
*****Significantly different from PRM, $P < 0.01$.
******Significantly different from PRM, $P < 0.001$.
[a]PRM, n = 9.
[b]PM, n = 16; PRM, n = 9.
[c]PRM, n = 11.

of hypothalamic and reproductive hormones in the blood. All subjects performed less than 1 h of moderate-intensity exercise per week, but some of the subjects cycled to work. All women had normal resting electrocardiography and were nonsmokers, and none of the subjects in either group had been diagnosed with osteoporosis, cardiovascular disease, renal dysfunction, insulin resistance, diabetes, or hypercholesterolemia. The study was conducted in accordance with the guidelines contained in the Declaration of Helsinki and was approved by the local Ethics Committee of Copenhagen and Frederiksberg communities Region H (H-1-2012-150). The subjects were informed of any risks and discomforts associated with the experiments before giving their written, informed consent to participate in the study. Age, weight, height, body mass index, hormone levels, $\dot{V}O_{2max}$, body composition, and training compliance and intensity after 0 and 12 wk of training have previously been published for 22 of the subjects (27).

As the main aim of the study was to detect differences in the training response between PRM and PM women, all women were assigned to the training group. Furthermore, an inactive control group was not included because studies from our laboratory have shown that body composition, markers of bone health, physical performance, and blood glucose levels do not change significantly in middle-age women during 3–16 months of inactive lifestyle (12,19).

Study design. Three experimental days were conducted before and after 12 wk of training for all subjects. Immediately after the 12-wk training period, a subgroup (PM40) of 9 PM women continued training for another 40 wk with a reduced training frequency followed by two experimental days and one testing day. The subjects refrained from exercise for 24 h before all experimental and testing days, and PRM underwent the first experimental day during the first day of menses, as this period is reliably identified.

Experimental days. On the first day, subjects came to the laboratory between 8:00 and 10:00 a.m. after an overnight fast. After 10 min of rest in a supine position, a catheter (20 gauge, 32 mm) was inserted into the antecubital vein for collection of blood. To avoid interference due to blood stasis, blood samples were drawn 15 min after venous cannulation. Blood samples for analysis of HbA1c and sex hormones were collected in precoated Vacuette tubes at room temperature for a maximum of 4 h before analysis. Blood samples for analysis of bone turnover markers, glucose, and insulin were centrifuged at 4°C in EDTA-K2-coated Vacuette tubes at 1000*g* for 15 min. After centrifugation, plasma was collected and stored in 1.5-mL Eppendorf tubes at −20°C. Body composition and whole-body BMD was then determined from whole-body dual-energy X-ray absorptiometry (DXA) scanning (Prodigy, GE Medical Systems, Milwaukee, WI). After 20 min of rest, a muscle biopsy (100–200 mg) was obtained from the vastus lateralis muscle using the percutaneous needle biopsy technique (5).

On the second experimental day, the subjects reported to the laboratory to perform an incremental exercise test on a treadmill in which $\dot{V}O_{2max}$ was determined (Oxycon Pro, Intramedic, Denmark). The test protocol consisted of 4 min of walking at 4 $km \cdot h^{-1}$ and then 4 min of walking at 6 $km \cdot h^{-1}$ interspersed with 2 min of rest in the standing position. Then after 2 min of rest, the subjects completed 2 min of walking at 6 $km \cdot h^{-1}$ followed by an incremental test to exhaustion increasing the speed of the treadmill by 1 $km \cdot h^{-1}$ every minute until volitional fatigue. Two PRM women could not complete the incremental exercise test after the 12-wk training period because of ankle pain (both women had experienced ankle injuries before enrollment to the study), and one PM woman did not complete this test because of personal reasons.

On the third experimental day, the subjects performed a countermovement jump test (as an indication of rate of force development and force) and the Yo-Yo intermittent endurance test level-1 (YYIET-1) (2). After 15 min of low-intensity warm-up, they performed two vertical countermovement jumps with arm swing. The jumps were separated by 2 min of rest; if a difference by more than 2 cm was observed, a third jump was performed. The highest jump was reported. The YYIET-1 consists of repeated 2 × 20-m shuttle runs at a gradually increased speed controlled by prerecorded audio beeps. Each shuttle run was separated by a 5-s walking period. The score of the test was recorded as the distance covered at the point when a subject had failed twice to complete the shuttle run in the allocated time. The distance of the last incomplete shuttle run was not included. Because of ankle injuries obtained before this study in two subjects and the absence of three others on the day of YYIET-1 and countermovement jump height testing after the 12-wk training period, 16 PM and 9 PRM women completed both tests and were included in the study.

Exercise training. The subjects were instructed to attend two weekly training sessions lasting 60 min each. During the 12-wk training period, the subjects in PM and PRM completed 1.8 ± 0.0 and 1.7 ± 0.1 training sessions a week (Table 2). To evaluate the ability of PM women to maintain their achieved benefits in bone health, body

TABLE 2. Training compliance and average training intensity for PM (n = 18) and PRM (PRM, n = 12) women during a 12-wk period with floorball training and for a subgroup of PM women (PM40, n = 9) for additional 40 wk.

	Training Sessions		Average Time per Session (min)	Total Training Time (% of HR_{max}), Pct.						
	Per Week	Total		<60%	61%–70%	71%–80%	81%–85%	86%–90%	91%–95%	96%–100%
12 wk										
PM	1.8 ± 0.0	21.1 ± 0.6	54.6 ± 0.7	26.0 ± 4.4	22.0 ± 1.7	21.0 ± 1.1	10.7 ± 1.1	10.0 ± 1.3	7.6 ± 1.6	2.7 ± 1.0
PRM	1.7 ± 0.1	20.1 ± 1.0	53.8 ± 0.9	25.8 ± 4.1	20.4 ± 1.0	19.8 ± 1.0	11.2 ± 0.7	12.2 ± 1.1	9.0 ± 2.1	1.6 ± 0.5
40 wk										
PM40	1.1 ± 0.1	44.1 ± 3.8	53.8 ± 0.8	16.7 ± 2.7	23.3 ± 1.9	25.5 ± 1.9	13.3 ± 1.0	14.1 ± 2.3	6.7 ± 1.7	0.3 ± 0.1

Values are expressed as means ± SEM. HR_{max}, maximal heart rate.

composition, and physical performance after 12 wk of training, a subset of PM women (PM40, $n = 9$) continued training for additionally 40 wk with an average of 1.1 ± 0.1 training sessions a week.

Each training session started with ~30 min warm-up consisting of low-intensity floorball drills followed by ~30 min of floorball play in 4- to 6-min intervals playing three against three on a 12 × 20-m court separated by 1–3 min of recovery. The subjects wore heart rate monitors (TEAM2 Wearlink+; Polar, Kempele, Finland) during each training session. Training compliance and intensity are presented in Table 2.

Measurements and analyses. Maximal heart rate was determined as the highest value obtained during the incremental treadmill test or any of the training sessions. Total lean leg mass and total leg BMD from the DXA scanning were determined for both the right and left leg from the area distally and laterally to the line between the iliac crest and ischial tuberosity. The precision error of the DXA scanner has previously been reported to be between 0.6% and 1.8% (30), which is in line with observations from our own laboratory. All analyses were performed by the same investigator. Plasma concentrations of CTX-1, P1NP, and total N-Mid OC were analyzed by a fully automated immunoassay system (iSYS; Immunodiagnostic Systems Ltd., Boldon, England) by method of chemiluminescence. The intermediary precision coefficient of variation ranged from 8% to 10%, and all three bone turnover marker analyses are accredited according to the ISO-15189. All analyses were run using the same batch of the assays for CTX-1, P1NP, and OC. P1NP and CTX-1 were chosen as they are the bone turnover markers recommended by the Working Group on Bone Turnover Markers to be used as reference markers and measured by standardized assays in observational and intervention studies (37). Plasma glucose concentrations were measured using a gluco-quant Glucose/HK kit (Roche Diagnostics GmbH, Mannheim, Germany) on Roche Hitachi P800/917 analyzer, and insulin concentrations were measured using an immunoassay kit (ELISA; DAKO, Glostrup, Denmark). Blood samples were analyzed by an automatic analyzer using HPLC for HbA1c, enzymatic kits for estradiol (Modular P-Module), progesterone, and testosterone (Modular E-Module) by using competitive electrochemiluminescence immunoassay and follicle-stimulating hormone and luteinizing hormone by using sandwich electrochemiluminescence immunoassay (Modular E-Module). We were unable to collect blood from one PRM women, and because of technical difficulties, CTX-1, P1NP, and OC were not analyzed for one of PM women.

Protein expression and muscle characteristics. The western blot analysis was used to determine protein expression, as previously described (26). Briefly, ~3 mg dry weight of the biopsy was homogenized in homogenization buffer, and the protein concentration of the lysate samples was determined (assayed in triplicate; only a coefficient of variation of less than 5% was accepted). Lysate proteins were separated using sodium dodecyl sulfate gels (Bio-Rad Laboratories, Hercules, CA) and transferred to PVDF membranes (Immobilion Transfer Membrane; Millipore, Billerica, MA). The membranes were incubated with primary polyclonal antibodies against glucose transporter type 4 (1:5000, glucose transporter type 4 [GLUT-4]; Thermo Fisher Scientific Inc., Rockford, IL), cytochrome *c* oxidase 4 (COX4, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), citrate synthase (CS, 1:3000; Abcam, Cambridge, UK), phosphofructokinase (PFK, 1:2000; Santa Cruz Biotechnology), mammalian target of rapamycin (mTOR, 1:1000; Cell Signaling Technology, Danvers, MA), myostatin (1:100; Santa Cruz Biotechnology), actin (1:2000; Sigma-Aldrich, Brøndby, Denmark), SR Ca^{2+}-ATPase 1 (1:10,000; SERCA 1; Thermo Fisher Scientific Inc.), and SERCA 2 (1:5000; Santa Cruz Biotechnology). Secondary antibody horseradish peroxidase–conjugated goat antirabbit (actin, CS, GLUT-4, mTOR, and myostatin), goat antimouse (COX4, PFK, and SERCA 1), and rabbit antigoat (SERCA 2) was used for detection. The protein content was expressed in arbitrary units (AU) relative to mixed human skeletal muscle standard samples run on each gel. Equal amounts of total protein were loaded for each sample, and samples from PM and PRM were distributed evenly across the gel. All samples were run simultaneously, and the sample from before training was placed adjacent to the sample after training for each subject. Because of insufficient amount of muscle tissue in biopsies from three PRM women, analysis of protein expression was not conducted for these subjects but for the remaining 27 subjects. A biopsy was not taken from one of the subjects in PM40 after the 40-wk training period because of personal reasons.

Muscle fiber cross-sectional area, fiber type distribution, and capillarization were analyzed using a staining protocol procedure previously described (25). The embedded muscle samples were cut using a cryostat, and transverse sections 8 μm in thickness were placed onto glass slides. To verify the cross-sectional orientation of the individual muscle fiber, multiple samples were cut and examined under light microscopy until a cross section of desirable size, orientation, and uniform polygonal appearance was visible. Only areas without artifacts or tendency to longitudinal cuts were analyzed. Staining targets were visualized pair wise. First, capillaries and myofiber type IIA were visualized using biotinylated Ulex europaeus agglutinin I lectin (1:100; VECTB-1065, VWR; Bie and Berntsen, Herlev, Denmark) and a monoclonal antibody (1:200; SC-71, Hybridoma Bank, Iowa City, IA), respectively. Second, myofiber borders were visualized using an antibody against laminin (1:500; DAKO Z0097, Denmark) together with myosin heavy chain (1:1000; Sigma-Aldrich M8421, Denmark) added for distinction of myofiber type I. Specific secondary antibodies (order listed: Streptavidin/FITC [1:200; DAKO F0422], Alexa-555 donkey antimouse [1:1000; Invitrogen, A31570, Life Technologies Denmark, Nærum, Denmark], Alexa-350 goat antirabbit [1:1000; Invitrogen, P10994], and Alexa-488 donkey antimouse [1:1000; Invitrogen, A21202]) were applied to each primary antibody. Specificity of the

staining was assessed by single staining and by staining without the primary antibody. Three individual muscle fiber types were identified as type I (green), type IIA (red), and type IIX (unstained/black) (6). Visualization was performed on a computer screen using a light microscope (Carl Zeiss, Germany), and all morphometric analysis were performed using a digital analysis program (ImageJ, NIH ImageJ, Bethesda, MD). Two or more separate sections of a cross section were used for analysis, and the cross-sectional area was assessed by manually drawing the perimeter around each selected section. The number of muscle fibers and capillaries within each section was counted, and capillary supply was subsequently expressed as capillaries per fiber (C:F-ratio) and capillary density (number per square millimeter). The mean fiber area was assessed by manual drawing of the perimeter of each muscle fiber. All analysis was conducted manually by the same blinded investigator.

Muscle samples from 12 subjects met the criteria of analysis. For the analysis of fiber area, fiber type distribution, and capillarization, 161 ± 12 and 140 ± 15 muscle fibers were analyzed per biopsy in PM (n = 7) and PRM (n = 5), respectively, in which 230 ± 17 and 188 ± 22 capillaries were identified.

Statistical analysis. Differences in baseline characteristics and training compliance and intensity were assessed with an unpaired samples Student's t-test. To investigate both intergroup differences and effects of the 12-wk training period, a two-way repeated-measures ANOVA was used. Pairwise differences were identified using Tukey's honestly significant difference *post hoc* procedure. Differences before and after the 40-wk training period in PM40 were assessed with a paired samples Student's t-test. The significance level was set at $P < 0.05$, and data are expressed as mean ± SEM.

RESULTS

Training compliance and heart rate. During the 12-wk training period, the subjects in PM and PRM completed 1.8 ± 0.0 and 1.7 ± 0.1 training sessions a week (Table 2). PM40 completed 1.1 ± 0.1 training sessions a week during the additional 40-wk training period. PM, PRM, and PM40 had a heart rate more than 85% of their maximum for more than 20% of the time during the training sessions.

Body composition. Before the training period, body fat percentage was higher ($P < 0.05$) in PM compared with PRM, whereas there was no intergroup difference in lean body and total lean leg mass (Table 1). After the 12-wk training period, PM had decreased ($P < 0.001$) body fat percentage by 2.2% ± 1.8% (38.6% ± 1.7% before vs 36.4% ± 1.9% after, n = 18), whereas there was no change in PRM (31.5% ± 2.3% before vs 31.1% ± 2.3% after, n = 12). After the 12-wk training period, both PM and PRM had increased ($P < 0.05$) lean body mass by 3.6% ± 0.5% and 1.6% ± 0.9% (41.1 ± 1.2 kg before vs 42.6 ± 1.3 kg after and 43.3 ± 1.5 kg before vs 44.0 ± 1.5 kg after, n = 18 and 12, respectively). After the 12-wk training period, PM had increased ($P < 0.05$) total lean leg mass by 4.6% ± 3.1% (14.7 ± 0.5 kg before vs 15.4 ± 0.7 kg after, n = 18), whereas it did not change in PRM (15.9 ± 0.6 kg before vs 16.4 ± 0.6 kg after, n = 12).

Physiological and functional capacity. There were no group differences in $\dot{V}O_{2max}$, YYIET-1, and countermovement jump height between PM and PRM before the training period (Table 1). After the 12-wk training period, PM and PRM had increased ($P < 0.05$) $\dot{V}O_{2max}$ by 4.4% ± 1.5% and 5.1% ± 2.4% (2311 ± 97 mL $O_2 \cdot min^{-1}$ before vs 2411 ± 101 mL $O_2 \cdot min^{-1}$ after and 2222 ± 134 mL $O_2 \cdot min^{-1}$ before vs 2324 ± 124 mL $O_2 \cdot min^{-1}$ after, n = 18 and 9, respectively). After the 12-wk training period, the distance covered in YYIET-1 for PM and PRM was 42.5% ± 7.9% and 40.6% ± 7.2% longer ($P < 0.001$, 713 ± 89 m before vs 1000 ± 120 m after and 1098 ± 234 m before vs 1404 ± 223 m after, n = 16 and 9, respectively). Countermovement jump height did not change during the 12-wk training period in either group (25.1 ± 0.9 cm before vs 26.6 ± 1.1 cm after and 30.8 ± 1.4 cm before vs 31.8 ± 1.3 cm after, n = 16 and 9, respectively).

Plasma bone turnover markers and BMD. Before the training period, CTX-1 was higher ($P < 0.01$) in PM compared with PRM, whereas there was no intergroup differences in P1NP, OC, total leg, and whole-body BMD (Fig. 1 and Table 1). The plasma bone resorption marker CTX-1 did not change during the 12-wk training period in either group (0.73 ± 0.08 $\mu g \cdot L^{-1}$ before vs 0.78 ± 0.08 $\mu g \cdot L^{-1}$ after and 0.43 ± 0.04 $\mu g \cdot L^{-1}$ before vs 0.50 ± 0.08 $\mu g \cdot L^{-1}$ after, n = 17 and 11, respectively; Fig. 1A). In PM, the bone formation marker P1NP in plasma increased by 40% ± 20% ($P < 0.001$, 54.7 ± 4.9 $\mu g \cdot L^{-1}$ before vs 74.3 ± 6.0 $\mu g \cdot L^{-1}$ after, n = 17; Fig. 1B) after the 12-wk training period, whereas no change was detected in the PRM (40.8 ± 3.0 $\mu g \cdot L^{-1}$ before vs 47.1 ± 4.1 $\mu g \cdot L^{-1}$ after, n = 11). The plasma bone turnover marker OC did not change in PM (22.3 ± 3.2 $\mu g \cdot L^{-1}$ before vs 24.0 ± 2.4 $\mu g \cdot L^{-1}$ after n = 17; Fig. 1C) during the 12-wk training period, whereas it increased with 24% ± 26% ($P < 0.05$, 14.9 ± 1.0 $\mu g \cdot L^{-1}$ before vs 18.4 ± 1.6 $\mu g \cdot L^{-1}$ after, n = 11) in PRM.

After the 12-wk training period total leg BMD in PM and PRM increased 1.8% ± 0.4% and 2.2% ± 0.9% ($P < 0.01$, 1.26 ± 0.02 $g \cdot cm^{-2}$ before vs 1.28 ± 0.02 $g \cdot cm^{-2}$ after and 1.27 ± 0.02 $g \cdot cm^{-2}$ before vs 1.30 ± 0.03 $g \cdot cm^{-2}$ after, n = 18 and 12, respectively; Fig. 1D), whereas no changes were detected in whole-body BMD (1.19 ± 0.02 $g \cdot cm^{-2}$ before vs 1.20 ± 0.02 $g \cdot cm^{-2}$ after and 1.18 ± 0.02 $g \cdot cm^{-2}$ before vs 1.19 ± 0.02 $g \cdot cm^{-2}$ after, n = 18 and 12, respectively, Table 1).

Blood glucose regulation. There were no group differences in plasma HbA1c, fasting glucose, and insulin and muscle expression of GLUT-4 between PM and PRM before the training period (Table 3). After the 12-wk training period, fasting plasma glucose increased 5.9% ± 2.1% ($P < 0.05$, 5.29 ± 0.14 $mmol \cdot L^{-1}$ before vs 5.59 ± 0.17 $mmol \cdot L^{-1}$ after, n = 18) in PM but did not change in PRM (5.19 ± 0.15 $mmol \cdot L^{-1}$ before vs 5.48 ± 0.13 $mmol \cdot L^{-1}$ after, n = 11).

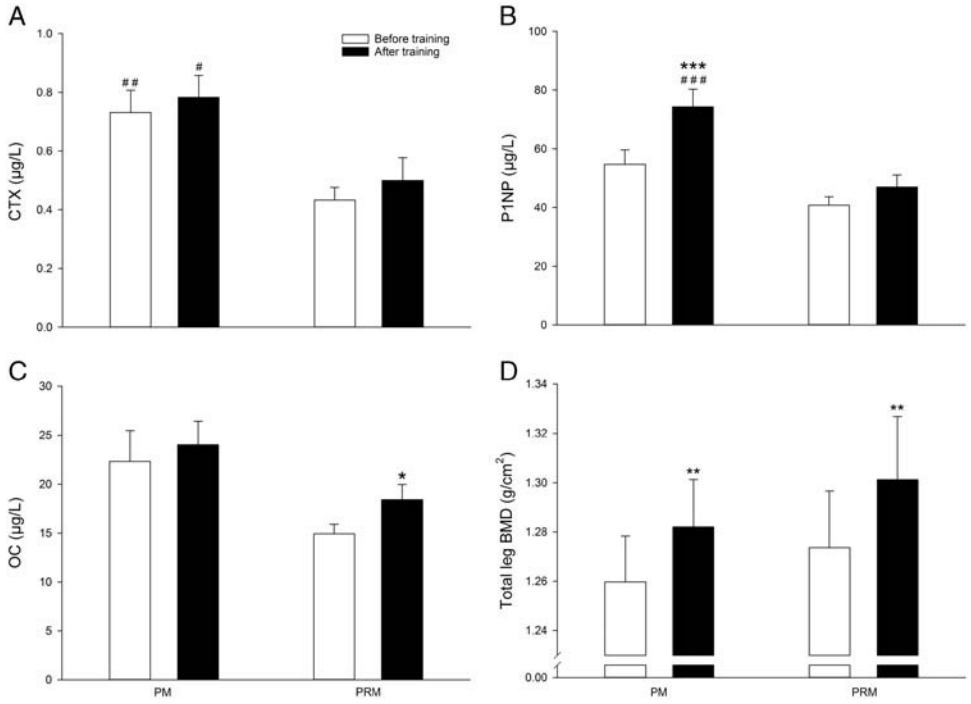

FIGURE 1—CTX-1 (A), P1NP (B), OC (C), and total leg BMD (D) before (*open bars*) and after (*filled bars*) 12 wk of floorball training for PM (n = 17–18) and PRM women (n = 11–12). Values are expressed as means ± SEM. Significantly different from before training, *P < 0.05, **P < 0.01, and ***P < 0.001. Significantly different from PRM, #P < 0.05, ##P < 0.01, and ###P < 0.001.

Metabolic and cell growth regulation. Before the training period, mTOR was higher (P < 0.01) and actin lower (P < 0.001) in PM compared with PRM (Table 3). The expression of mTOR and actin did not change during the 12-wk training period in either group. The expression of muscle PFK did not change in PM (0.83 ± 0.07 AU before vs 0.80 ± 0.06 AU after, n = 18) but decreased 23% ± 14% (P < 0.01, 0.93 ± 0.09 AU before vs 0.74 ± 0.11 AU after, n = 9) in PRM. The expression of COX4, CS, myostatin, SERCA 1, and SERCA 2 did not change with training in either group, and no intergroup differences were found (Table 3).

Muscle composition and capillaries. There was no group differences in fiber type distribution between PM and PRM neither before nor after the 12-wk training period. Before the training period, the area of MHC-IIX fibers was lower (P < 0.05) in PM compared with PRM, whereas no intergroup difference was observed in the fiber area of other fiber types. In PM, the distribution of muscle fibers was not different after compared with before the 12-wk training period, whereas in PRM, the percentage of type IIX fibers increased 5.6% ± 2.9% (P < 0.05, 4.0% ± 0.9% before vs 9.6% ± 6.5% after, n = 5). In PM, mean fiber

TABLE 3. Blood levels of glucose and insulin, muscle expression of glycolytic and oxidative enzymes, and markers of muscular growth and capillary density in PM (PM, n = 18) and PRM (PRM, n = 9) women before and after a 12-wk period with floorball training.

	PM (n = 18)		PRM (n = 9)	
	Before	After	Before	After
HbA1c (mmol·L^{-1})[a]	6.14 ± 0.12	6.28 ± 0.14	6.15 ± 0.14	6.10 ± 0.14
Glucose (mmol·L^{-1})[a]	5.29 ± 0.14	5.59 ± 0.17*	5.19 ± 0.15	5.48 ± 0.13
Insulin (pmol·L^{-1})[a]	32.6 ± 4.6	34.0 ± 4.5	37.8 ± 5.4	32.8 ± 2.1
GLUT-4 (AU)	0.26 ± 0.05	0.27 ± 0.05	0.38 ± 0.05	0.43 ± 0.05
PFK (AU)	0.83 ± 0.07	0.80 ± 0.06	0.93 ± 0.09	0.74 ± 0.11**
mTOR (AU)	1.30 ± 0.09****	1.40 ± 0.10****	1.78 ± 0.14	1.88 ± 0.07
Myostatin (AU)	0.47 ± 0.08	0.43 ± 0.08	0.45 ± 0.09	0.48 ± 0.07
Actin (AU)	2.11 ± 0.17*****	1.93 ± 0.13***	1.12 ± 0.13	1.37 ± 0.16
Capillary density (mm^{-2})[b]	369 ± 11	421 ± 17	359 ± 24	366 ± 37
Capillary-to-fiber ratio[b]	1.50 ± 0.06	1.38 ± 0.09	1.30 ± 0.08	1.39 ± 0.05

Values are expressed as means ± SEM.
*Significantly different from before training, P < 0.05.
**Significantly different from before training, P < 0.01.
***Significantly different from PRM, P < 0.05.
****Significantly different from PRM, P < 0.01.
*****Significantly different from PRM, P < 0.001.
[a]PRM, n = 11.
[b]PM, n = 7; PRM, n = 5.

area and area of fiber type I and IIA was not different after compared with before the 12-wk training period, whereas the area of type IIX fibers increased 32.5% ± 9.2% ($P < 0.05$, 1525 ± 65 μm^2 before vs 2020 ± 271 μm^2 after, $n = 7$).

Before the training period, capillary density and capillary-to-fiber ratio was not different between groups, and they did not change during the 12-wk training period in neither PM nor PRM (Table 3).

Effect of additional 40 wk of training with a reduced frequency on bone, body composition, skeletal muscle, and glucose control. From the end of the 12-wk training period to the end of the additional 40-wk training period, plasma bone turnover markers and total leg BMD in PM40 did not change, whereas whole-body BMD decreased 1.2% ± 0.5% ($P < 0.05$, 1.181 ± 0.028 $g \cdot cm^{-2}$ before vs 1.166 ± 0.027 $g \cdot cm^{-2}$ after, $n = 9$). Plasma HbA1c decreased 7.2% ± 1.6% ($P < 0.01$, 6.31 ± 0.17 $mmol \cdot L^{-1}$ before vs 6.22 ± 0.15 $mmol \cdot L^{-1}$ after, $n = 9$), and the expression of muscle GLUT-4 increased 88% ± 39% ($P < 0.05$, 0.47 ± 0.08 AU before vs 0.77 ± 0.11 AU after, $n = 8$). Fasting plasma glucose and insulin was not different after compared with before the 40-wk training period. Body fat percentage increased 2.2% ± 0.5% ($P < 0.01$, 33.8% ± 3.0% before vs 35.9% ± 2.6% after, $n = 9$), and lean body mass decreased 3.3% ± 0.8% ($P < 0.01$, 41.9 ± 1.5 kg before vs 40.5 ± 1.4 kg after, $n = 9$) after the 40-wk training period. There was no difference in total lean leg mass after compared with before the 40-wk training period, and no differences in $\dot{V}O_{2max}$, YYIET-1, and countermovement jump height were detected after the training period (Table 4).

DISCUSSION

The current study showed that 12 wk of floorball training twice a week induced similar increases in performance, lean body mass, and total leg BMD in PRM and recent PM women. In addition, only in PM women was the percentage of body fat reduced and plasma P1NP increased with the intervention, whereas plasma OC was increased in PRM women. In a subgroup of PM women who performed 40 additional weeks of floorball training with a reduction in training frequency, these improvements were maintained, and the level of blood HbA1c was lowered and expression of muscle GLUT-4 protein was higher than after 12 wk of training.

TABLE 4. Change in bone turnover markers, blood levels of glucose and insulin, skeletal muscle GLUT-4, body composition, and performance in PM women (PM40, $n = 9$) after additional 40 wk of floorball training with reduced training frequency (weeks 12–52).

	PM40 ($n = 9$)	
	Change with Additional 40 wk of Training	Effect of Training
CTX-1 ($\mu g \cdot L^{-1}$)	−0.08 ± 0.09	$P = 0.438$
P1NP ($\mu g \cdot L^{-1}$)	−11.3 ± 5.1	$P = 0.116$
OC ($\mu g \cdot L^{-1}$)	−2.8 ± 0.8	$P = 0.080$
HbA1c ($mmol \cdot L^{-1}$)	−0.47 ± 0.11**	$P = 0.003$
GLUT-4 (arbitrary units)[a]	0.29 ± 0.13*	$P = 0.044$
Glucose ($mmol \cdot L^{-1}$)	−0.13 ± 0.10	$P = 0.243$
Insulin ($pmol \cdot L^{-1}$)	−1.3 ± 2.2	$P = 0.584$
Whole-body BMD ($g \cdot cm^{-2}$)	−0.015 ± 0.006*	$P = 0.048$
Total leg BMD ($g \cdot cm^{-2}$)	0.002 ± 0.015	$P = 0.876$
Body fat (%)	2.2 ± 0.5**	$P = 0.003$
Lean body mass (kg)	−1.4 ± 0.4**	$P = 0.005$
Total lean leg mass (kg)	−0.6 ± 1.0	$P = 0.563$
$\dot{V}O_{2max}$ (mL $O_2 \cdot min^{-1}$)	2.8 ± 55.5	$P = 0.961$
YYIET-1 (m)	4.4 ± 73.8	$P = 0.953$

Values are expressed as means ± SEM.
*Significant difference before and after additional 40 wk of training, $P < 0.05$.
**Significant difference before and after additional 40 wk of training, $P < 0.01$.
[a]$n = 8$.

It has been suggested that estrogen and other hormones associated with the PRM phase are essential for training-induced adaptations in skeletal muscle and bone (32,38). In the current setting, the 12-wk training period was associated with similar improvements in total lean leg mass, lean body mass, body fat percentage, and maximal oxygen uptake and intermittent performance in the two groups of women. The large effect on Yo-Yo performance (~40%) as opposed to the less pronounced changes in $\dot{V}O_{2max}$ (~5%) may reflect the intermittent nature of floorball. This difference could somewhat reflect familiarization to performing the Yo-Yo test, but because the tests were separated by 12 wk, this effect may be limited. The similar adaptations in the two groups suggest that the hormonal milieu associated with the initial PM phase does not blunt the aerobic and anaerobic training adaptations to intense intermittent exercise. In agreement, the improvements in body composition and performance are comparable with that observed in younger untrained PRM women who performed 16 wk of football training (3,14). Taken collectively, it appears that recent PM women can benefit from intense intermittent exercise to a similar extent as PRM women. This effect of intermittent exercise is highly relevant as menopause is normally associated with an increased loss of muscle mass and strength (22).

It is commonly observed that a loss of bone mass, muscle mass, and strength occurs after menopause (11,22). Thus, it was a striking finding that elevated plasma P1NP concentrations, total leg BMD, total lean leg mass, $\dot{V}O_{2max}$, and physical performance were maintained in PM women for additional 40 wk with a reduced training frequency to only 1.1 training sessions a week. This indicates that floorball training is particularly beneficial in maintaining bone and muscle mass in the legs and preserving physical performance in recent PM women. The importance of maintaining bone health in this population is underlined by the finding that whole-body BMD decreased by 1.2% ± 0.5% during the additional 40 wk of training. Although floorball training shows specifically beneficial in preserving leg bone health, this finding, furthermore, implies that floorball training once a week is not sufficient to maintain whole-body bone health in PM women. Another study had early PM women completing a 12-month whole-body exercise training program with various types of endurance and strength exercises more than twice a week resulting in only 1.3% increase in BMD (17). Although BMD was somewhat increased, this indicates, together with our findings, that improving BMD in this population over the course of time is a challenge, and that the combination of the right exercise activities with

suitable training frequency and volume is important in continuously upholding bone health in this group. It is also noteworthy that the subjects in the present study were able to maintain $\dot{V}O_{2max}$ with only 1.1 training session a week. Because a low level of $\dot{V}O_{2max}$ is associated with an increased risk of lifestyle diseases and mortality, maintaining $\dot{V}O_{2max}$ in PM women with only one training session per week is an important finding considering that most governing bodies recommend five training sessions per week or more for health-related benefits. Albeit, the results from this study indicate that health parameters can be improved with as little as one to two training sessions a week for this population. Further research is needed to investigate the minimal exercise intensity and volume requirements that induce health adaptations.

Improvement of bone formation in PM women is of critical importance because bone resorption is upregulated during and after menopause with no changes in bone formation, thereby resulting in an overall increased bone loss (33). In congruence, in the present study, the plasma resorption marker CTX-1 was higher in PM compared with PRM women with no differences in the bone formation marker P1NP prior to training. The osteogenic response to exercise is typically induced by a high strain rate and by various muscle and ground reaction forces targeting the bone (18). As a decline in the osteogenic responsiveness to mechanical load has been shown to occur with menopause (4), we hypothesized that the improvements in bone formation markers and BMD with training would be more pronounced in the PRM women. By contrast, 12 wk of floorball training increased the level of circulating P1NP in the PM women only and, with no increase in CTX-1, elicited a similar increase in total leg BMD in the PRM and PM women. BMD in younger PRM women did not change with 14 or 16 wk of football training (12,14). This discrepancy between the current findings and that of the effects of football training (12,14) is interesting as the training frequency and leg BMD at baseline in the present and the two aforementioned studies are comparable. It may reflect different effects on the legs as ground reaction forces would be expected to be higher in floorball as a consequence of the harder ground surface (floor vs grass). The difference may also be related to differences in the regulation of bone turnover in young and middle-age women as it has been shown that advancing age in women is associated with a loss of estrogen receptor regulation and diminution of signal transduction in osteoblasts (1). Taken collectively, the present findings demonstrate that floorball training twice a week was effective in promoting an increase in BMD in middle-age women irrespective of menopausal status, indicating that the osteogenic responsiveness to high ground reaction forces is not affected in the early phase after menopause. Because the femoral neck is a common site for osteoporosis-related fractures, measurements of mineral density at this specific site would be of interest in this population in future studies.

Another interesting finding in the present study was that HbA1c was lower after 52 wk of training, indicating that the intense intermittent exercise was effective in preserving a healthy blood glucose profile because HbA1c is a diagnostic tool for diabetes. Other studies have also shown reduced HbA1c in healthy women after a 5-month training period, but with training four to five times a week (36). The lack of change in HbA1c after 12 wk of training in the current study stands out because the subjects did undergo improvements on total leg BMD and bone formation markers with this intervention. This could indicate that floorball training twice a week is more suitable for improving bone health than blood glucose control, which could be explained by the numerous accelerations and changes of direction that occurs during floorball. Notably, this lack of change in HbA1c after 12 wk of training may be related to an insufficient training frequency and/or the low baseline level in these women as a high level has been shown to be predictive for both a short (1 month) and long-term (9 months) training-induced lowering of HbA1c (9). The observed higher expression of skeletal muscle GLUT-4 with 52 wk of floorball training indicates that an improved muscle glucose uptake capacity in skeletal muscle may have been one mechanism underlying the lowering of the HbA1c levels. Considering also that skeletal muscle mass in middle-age women account for ~30% of the total body mass (15) and GLUT-4 is the most abundant glucose transporter in muscle cells and the content of this transporter is positively correlated with muscle glucose uptake (13). The lack of change in muscle GLUT-4 after 12 wk, however, indicates that a prolonged period or higher training frequency is needed to increase the level of glucose transporters in skeletal muscle with intense intermittent exercise. The present data indicate the importance of physical activity and suggest that one to two training session a week containing intense periods can improve blood glucose regulation and preserve bone health, body composition, and physical performance in recent PM women.

The absence of estrogen is associated with a reduction in type II muscle fibers (24), and it has also been reported that PM women have fewer type II muscle fibers and lower cross-sectional area compared with younger PRM women (20). Although based on cross-sectional observations, the present data do indicate that fiber type distribution is not altered during the first years after the menopausal transition and that the number and size of type II fibers are maintained in recent PM women. It has been suggested that the loss of muscle strength with reduced estrogen levels is caused by a reduction of the intrinsic qualities of muscle fibers rather than reduced muscle size (21), which is in accordance with the present findings. Despite the similar size of the muscle fibers, a lower expression of mTOR was detected in the PM women. This protein is essential for muscle metabolism and a central regulator of skeletal muscle hypertrophy (7). Estrogen depletion has been shown to decrease mTOR activity (28), and the lower mTOR levels in the recent PM women compared with PRM women in the present study indicates that anabolic signaling is affected shortly after the menopausal transition. Nevertheless, total lean leg and body mass

in the PM women of this study increased, amplifying the effectiveness of floorball training in this population.

In summary, only approximately two sessions a week with floorball training for 12 wk can improve bone health, body composition, and physical performance in both PRM and PM women of similar age, with only few differences in the training response between these groups of women. These observations suggest that estrogen and other hormones associated with the PRM phase are not essential for training-induced changes and that PM women are as responsive to exercise training on these parameters as PRM women of similar age. The adaptations can be maintained to a large extent, and blood glucose control can even be improved with a reduced frequency of training in PM women, which may be related to the periods of intense actions during floorball. These results show that floorball training is effective in improving bone, muscle, and aerobic health in recent PM women, even with only one to two training sessions per week.

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