3rd December 2021

Study protocol with SAP

The supervised multimodal training in the present 19-weeks study was offered to postmenopausal healthy women in a local community. The outcomes for the training group (MMT) were compared with a sedentary control group (CON). Training was offered twice weekly for one hour, and the average attendance rate of MMT participants over the 19wk had to be > 1 hour weekly.

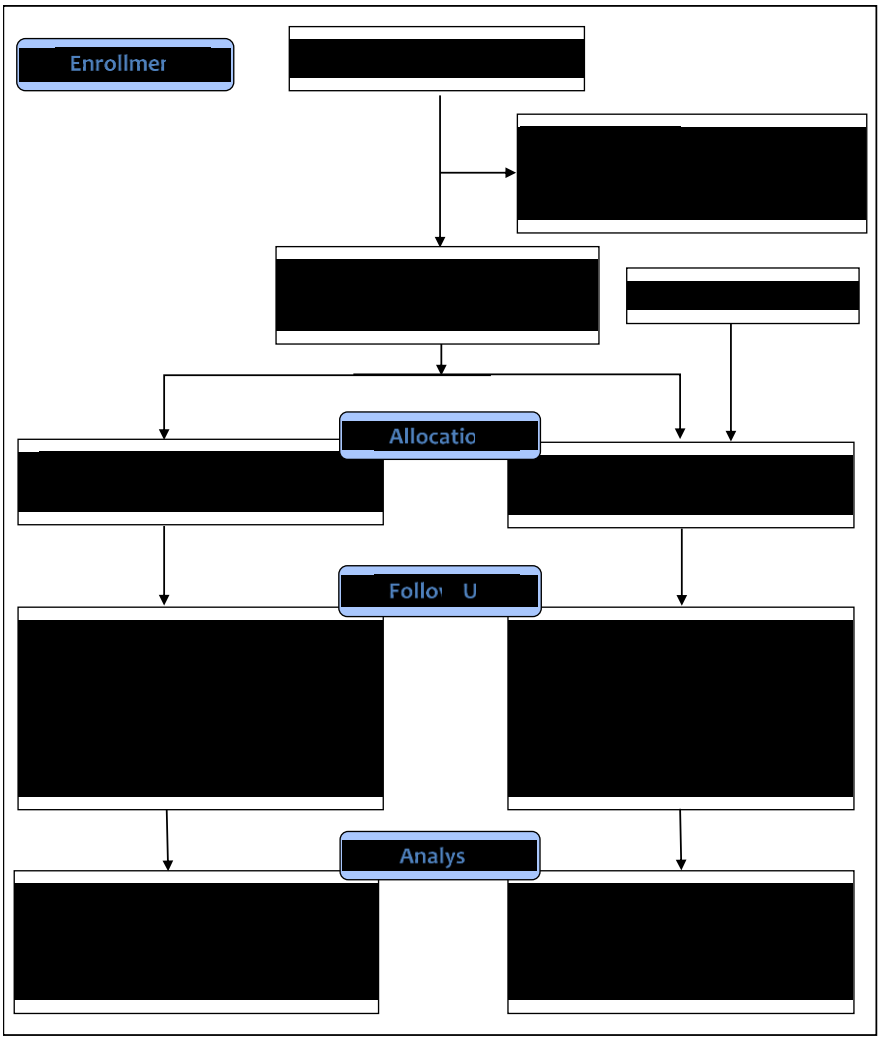
To collect baseline (BASE) data, all participants showed up in the laboratory at three occasions prior to the intervention. On the first visit, resting blood samples were collected to evaluate the concentration of bone turnover markers (BTM), and body weight, height, body composition, bone mineral density (BMD) and bone mass were measured. On the second visit, a dynamic balance test (four square step test) and a functional muscle strength and power test (jump-and-reach test) were performed. On the third visit, the aerobic capacity was evaluated by assessment of VO2-max. After three weeks (3wk) of training, resting blood samples were collected again. After 19wk of training, all assessments were repeated and compared with baseline, and MMT and CON were finally compared.

Blood sample collection, DXA-scanning, and VO2-max tests were carried out at the Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark. The training and testing of vertical jump height, as well as dynamic balance, were performed at the health promotion training initiative in the Copenhagen area, Denmark. The blood samples were analyzed at the Department of Clinical Biochemistry, Rigshospitalet, Glostrup Hospital, Denmark.

Participants

Healthy, sedentary postmenopausal women aged below 70 years were eligible to participate in the present study. Inclusion criteria were: non-smoking and body mass index (BMI) <30 kg/m^2. Exclusion criteria were: T-score < -3 SD in the lumbar spine or hip; Z-score > 1.5 SD); use of hormone therapy, medical treatment, or supplements that affect bone metabolism; previous or current medical condition affecting bone health; engagement in regular and systemic weight-bearing training or strength training during the preceding two years.

A flow diagram is shown below: Initially, twenty women were recruited to the training via an online advertisement and a local newspaper, but only 19 showed up for pre-testing. After a medical examination, one participant was excluded due to low BMD (T-score < -3 SD), and two were excluded due to high BMI (≥30 kg/m^2). In addition, one woman refrained from participating in the training, and thus, 15 participants were recruited to MMT. During the study, two participants in MMT dropped out due to personal reasons and at the end of the study four participants were excluded in the analyses as their average attendance to the program was less than one hour per week. CON consisted of 13 age-matched sedentary postmenopausal women (12 from a previous study plus the woman who refrained from participating in the training), and therefore 9 MMT and 13 CON was finally included in the data analyses.



CONSORT Flow Diagram

Participants' baseline characteristics, including maximum oxygen consumption (VO2-max), are shown in Table 1.

Table 1. Baseline characteristics of the participants

	MMT (n=9)	CON (n=13)	p-value
Age (years)	62.4 ± 3.8	58.7 ± 4.7	0.061
Height (cm)	166.9 ± 6.4	166.9 ± 5.2	0.989
Weight (kg)	72.8 ± 5.6	64.2 ± 10	0.022*
BMI (kg/m^2)	26.3 ± 2.2	22.9 ± 2.9	0.009*
Body Fat Percentage (%)	39.4 ± 3.1	33.7 ± 8.5	0.070
Total Fat Mass (kg)	28.7 ± 2.7	22.2 ± 8.6	0.043*
Total Lean Body Mass (kg)	41.8 ± 4.3	39.8 ± 3.4	0.233
VAT-mass (g)	793 ± 351.2	511 ± 434.8	0.123

VAT-volume (cm^3)	840.6 ± 372.2	541.7 ± 460.9	0.123
Total BMC (g)	2266.9 ± 206.5	2067.2 ± 300.2	0.100
BMD (g/cm^2)			
Whole-body	1.121 ± 0.073	1.046 ± 0.099	0.066
Lumbar spine	1.088 ± 0.137	1.014 ± 0.111	0.175
Right Femur neck	0.918 ± 0.092	0.834 ± 0.097	0.057
Left Femur neck	0.941 ± 0.088	0.823 ± 0.076	0.003*
Mean Femur neck	0.929 ± 0.089	0.829 ± 0.085	0.015*
Right Total Femur	0.947 ± 0.098	0.864 ± 0.091	0.056
Left Total Femur	0.949 ± 0.101	0.840 ± 0.091	0.016*
Mean Total Femur	0.948 ± 0.098	0.852 ± 0.090	0.028*
Right Trochanter	0.741 ± 0.087	0.683 ± 0.092	0.149
Left Trochanter	0.745 ± 0.091	0.661 ± 0.097	0.055
Mean Trochanter	0.743 ± 0.086	0.672 ± 0.093	0.084
Right Shaft	1.151 ± 0.126	1.046 ± 0.125	0.068
Left Shaft	1.149 ± 0.131	1.008 ± 0.121	0.017*
Mean Shaft	1.150 ± 0.126	1.027 ± 0.121	0.033*
BTM			
P1NP (µg/l)	52.8 ± 16.6	62.1 ± 21.3	0.298
OC (µg/l)	19 ± 6.5	28.9 ± 7.1	0.003*
CTX (ng/l)	331.2 ± 100.7	627.8 ± 253.8	0.001*
VO_2 max (ml/min/kg)	28.2 ± 3.6	30.5 ± 4.7	0.204

Values are given as means ± SD. BMI = body mass index, BMD = bone mineral density, BTM = bone turnover marker. *Significant difference between groups ($p<0.05$). Unpaired T-test.

Every participant was fully informed before giving her written informed consent to the procedures and potential discomfort associated with the study. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee of the Capital Region of Denmark, H-18044190.

Training program

The present feasibility study evaluated the osteogenic impact of a training concept already offered by the health promotion initiative in a local community.

According to the initiative, the training was offered as "evidence-based bone training" aimed upon enhancing musculoskeletal health. It was carried out twice a week, 60 minutes each session. The training concept included: 1) High- and odd-impact exercise including multi-directional games; 2) Progressive resistance training; 3) Balance and coordination training. The participants were engaged in intermittent gymnastics and small game sessions aimed at imposing osteogenic and diverse strain on the skeleton, mainly in the legs and arms. Thus, the training included various jumping exercises (e.g., counter-movement jump, from the floor to the bench, and from one bench to the floor), quick walking up and down on gymnastics

equipment, sprinting over short distances in different directions (in small games form). In addition, resistance exercises with power band and elastic bands, heavy exercise balls (medicine balls), and sandbags of 5-7 kg were applied. The training was performed intermittent and varied to ensure that the bones would not be desensitized, as it is seen in endurance training.

BMD and body composition

To test the BMD exclusion criteria at baseline as well as evaluate bone adaptation after 19wk of training, BMD (g/cm^2) of proximal femur (PF), lumbar spine (LS), and whole-body (WB) were assessed by Dual-energy X-ray Absorptiometry scanning (iDXA, Lunar Corporation, Madison, Wisconsin, USA) according to standard procedures. The regions of interest were determined by the encore software (encore software version 14.10.022, GE Medical Systems, Madison, United States). In addition, body composition was evaluated by the whole-body scan: body weight (BW, kg), BMI (kg/m^2), body fat percentage (%BF, %), total fat mass (FM, g), total lean body mass (LBM, g), visceral adipose tissue (VAT) mass (g) and volume (m^3), and total bone mineral content (BMC, g). The participants were asked to remove metal objects and empty their bladder prior to scanning.

Blood sampling & Biochemical analyses

The plasma concentration of BTM at BASE, after 3wk, and after 19wk of training were measured. After an overnight fast and without any vigorous activities in the previous 48 hours, participants showed up in the laboratory in the early morning. Blood samples were collected from the antecubital vein with a butterfly needle, then transferred to EDTA tubes and centrifuged immediately. The plasma fractions were put on dry ice. Eighteen ml of blood were taken from each participant per test day. Following each test, a sample was placed at -80◦C for future P1NP, OC, and CTX analysis, which were assessed by the Chemiluminescence method using a fully automated immunoassay system (iSYS, Immunodiagnostic Systems Ltd., Bolton, England). The assay performance expressed as inter-run variation coefficients were 8% for P1NP, 9% for OC, and 10% for CTX.

Training status

To estimate the participants' general training status, a progressive test of maximal oxygen uptake (VO2-max) (ml/kg/min) was performed on an electronic ergometer cycle (Monark 839E, Monark Exercise AB, Vansbro, Sweden) according to standard procedures. A breath-by-breath gas online analyzing system (Jaeger Oxycon Pro, VIASYS Healthcare, Höchberg, Germany) was connected to the participant, and a direct VO2-max measurement was conducted.

Dynamic balance

The "four square step test" (FSST) was performed to evaluate the dynamic balance [18] in MMT. The test requires a person to move systematically forward, sideward, backward, and sideward again over four narrow sticks circa 2.5 cm in diameter and 100 cm in length placed on the floor like a cross. The participants were requested to complete the sequence as fast as possible without touching the sticks. The participants started by facing the cross in the lower left quadrant, stepped forward over the stick into the next left quadrant, stepped sideward over the stick again into the upper right quadrant, and stepped backward over the stick into the lower right quadrant, then stepped sideward over the stick into the left quadrant where they started. Thus, they were moving in a clockwise direction. The floor in each square must be contacted by both feet, and the participants were asked to face forward for the whole sequence if possible. The participants were allowed to turn to step into the next square if needed. They started and finished in the same square, and the score were given in seconds (sec). The test was done twice, and the fastest time was used as the FSST score.

Functional muscle strength and power

The functional muscle strength and power were evaluated in MMT by a jump-and-reach test (vertical jump height) (Vertec Sports Imports, Hilliard, OH). First, the participants were taught how to perform a countermovement jump and how to displace the vanes that was placed above reaching height on a vertical stand. After thoroughly instruction and familiarization, the jump-and-reach test was performed. The maximum vertical jump height (cm) was determined by the difference between the participant standing reach height and the highest displaced vane. The greatest value out of three trials was taken as the result.

Statistical analysis

All of the statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 26.0, IBM Corp., Armonk, NY, USA). The standard descriptive statistics and unpaired T-test were used to describe and test the baseline characteristics of the participants. Repeated measurements ANOVA and general linear model with post hoc tests were used to test the effect of training on BTM, BMD, dynamic balance, and the jump-and-reach test. A p-value < 0.05 was considered significant.