ORIGINAL ARTICLE

Effects of 8-week increment aerobic exercise program on bone metabolism and body composition in young non-athletes

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Abstract

Purpose The effects of aerobic exercise on bone metabolism are still unclear. Thus, the main goal of this study was to explore if there was an effect of the short-term aerobic exercise program on the bone remodeling process and if there were sex differences in the effect of the training program on bone metabolism.

Methods Twenty-one participants (men and women) aged 20–23 performed an 8-week aerobic exercise program three times per week in 1-h sessions with increases in the exercise load every 2 weeks. Bone density, bone mineral content and concentration of markers of bone metabolism: osteocalcin, C-terminal procollagen type I peptide, pyridinoline, parathyroid hormone, osteoprotegerin, and the receptor activator of nuclear kappa B ligand by ELISA were measured at the start and at the end of the study, while changes in body composition were assessed by a bioelectric impedance analysis method 6 times during the study.

Results The aerobic exercise program increased the concentration of osteocalcin (11.34 vs 14.24 ng/ml), pyridinoline (67.51 vs 73.99 nmol/l), and the receptor activator of nuclear kappa B ligand (95.122 vs 158.15 pg/ml). A statistically significant increase in bone density at neck mean (1.122 vs 1.176 g/cm^3) and in bone mineral content at dual femur (33.485 vs 33.700 g) was found in women, while there was no statistically significant change at any site in men.

Conclusion 8 weeks of the aerobic exercise program with increment in intensity increased some of bone remodeling biomarkers and showed different effects for men and women.

Keywords Aerobic exercise · Bone turnover · Body composition · Biomarkers of bone metabolism

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Abbreviations

BIA	Bioelectric impedance analysis
BMI	Body mass index
LBM	Lean body mass
MBF	Mass of body fat
PBF	Percent of body fat
TBW	Total body water
MIN	Mineral content of the body
BMD	Bone mineral density
BMC	Bone mineral content
OC	Osteocalcin
PICP	C-terminal procollagen type I peptide
PYD	Pyridinoline
PTH	Parathyroid hormone
OPG	Osteoprotegerin
RANKL	Receptor activator of nuclear kappa B ligand
ELISA	Enzyme-linked immunosorbent assay
DF TOTAL	Dual femur total mean
L1-L4	Lumbar spine L1-L4

NM   Neck mean (mean from results of left and right femoral neck)

CV   Coefficient of variability

DXA   Dual X-ray absorptiometry

Introduction

There is strong evidence that regular exercise and physical activity play an important role in preserving bone health, mass, and strength at all stages of life (Santos et al 2017). In childhood and adolescence, regular exercise increases bone accrual and bone mass (Baxter-Jones et al 2008; Hind and Burrows 2007; Meyer et al 2013; Nogueria et al 2014) while for adults and for the elderly exercise can help prevent bone loss (Bolam et al 2013; Howe et al 2011; Kelley et al 2013). This beneficial effect of exercise on bone mass is observed in some athletes who practice certain types of sports, those that consists of high-impact, multi-directional movements, as evidenced by higher bone mass in athletes who compete in these types of sports compared to sedentary controls (Brahm et al 1997; Mantovani et al 2017; Rautainen et al 2014). The mechanical load applied to the bones during exercise evokes the process of bone remodeling to make the bones stronger and more apt to handle the increased stress applied by the exercise. The remodeling process includes bone resorption and formation and leads to an increased resistance to fracture. The best effect on bone mass and strength is achieved by impact-inducing activities (like jumping), while sports like swimming and bicycling could lead to bone loss (Tenforde and Fredericson 2011) if performed exclusively and not combined with impact-inducing exercise. A mechanical load applied to the bone by physical exercise should induce a greater strain to a bone than habitual everyday physical activity, to initiate an osteogenic response. The adaptation of the bone to a mechanical load is very localized, as shown in sports with unilateral loading, e.g. tennis, where differences in bone properties were found between the playing and the non-playing arm (Bass et al 2002). Bone formation depends on strain rates and the applied tension peak, as well as on the frequency of stimulation (Lombardi 2019). However, it remains unclear how much load is necessary and for how long should it be applied to induce bone remodeling (Kelley et al 2013; Santos et al 2017). In addition, there is evidence about different effects of different exercise models on bone metabolism (Guadalupe-Grau et al 2009; Santos et al 2017; Turner and Robling 2003). As Guadalupe-Grau et al (2009) concluded in their review article, the impact and resistance exercise showed best results for the improvement of bone mass, while aerobic training does not have such a potent effect as resistance exercise. A similar conclusion was made by Lombardi (2019) who compared the bone mineral density of athletes performing different sports. He found that adolescents and adults participating in high-volume endurance and non-weight bearing activities have lower bone density than subjects playing ball and power sports.

The measurement of biochemical bone turnover markers is an effective way to study the impact of exercise on bone remodeling, since changes in the concentration of molecules involved in the remodeling process (markers of bone resorption and markers of bone formation) are a very sensitive indicator of remodeling (Ooi and Sahrir 2018; Seibel 2005). They also have their limitations. Many bone metabolism biomarkers are not bone specific. Products of collagen metabolism are found in many connective tissues, not only in bone. Some of them have functions in energy metabolism and muscle activity (Dolan et al 2020). Also, biomarkers are systemic and cannot indicate bone metabolism activity at any particular site, while the effect of exercise on bones is very localized and site specific. There is also a question of the quick changes in the serum concentration of biomarkers of bone remodeling, which makes measurement very difficult regarding sample collection timing. Novel markers appeared in the last decade. MicroRNA are promising markers for bone health, as they were shown to promote osteogenic differentiation of mesenchymal stem cells (MSCs) by suppressing osteogenic inhibitors or by mediating major osteoblastic differentiation and signaling pathways. They are promising markers for the explanation of cellular processes in bone remodeling (Ka-Fai Cheng et al., 2019). Different types of exercise produce different changes in the concentration of bone metabolism markers. For example, an aerobic training program increased bone formation after 8 weeks of practice, while an anaerobic training program resulted in an overall increase in the bone remodeling process, or in the acceleration of bone metabolism (Lester et al 2009; Woitge et al 1998). The metabolic adaptation of bone turnover to exercise depends on age, gender, and the type of exercise performed (Vasikaran et al 2011; Dolan et al 2020). Combined-impact exercises, odd-impact or high-impact combined with resistance training, are recommended for maximizing bone mineral density at a young age (Xu et al 2016; Weaver et al 2016). Intermittent stimulation has better effects compared to continuous stimulation for the increase in bone formation (Santos et al 2017; Martin-St James and Caroll 2010). However, the literature on the influence of interval aerobic training with variations in intensity to the bone remodeling process is scarce (Hind and Burrows 2007; Kelley et al 2013). Aside from improvements in aerobic capacity, aerobic training might also induce changes in body composition, and contribute to health in general. Running is not as potent an osteogenic exercise as jumping, because of its repetitiveness, and it could cause the adaptation of bone cells to routine strain. However, the results on the osteogenic potential of running from literature are somewhat contradictory, as pointed out in the systematic

review by Garofolini and Taylor (2019): the outcome suggests that running may increase bone density among other benefits, but it depends on training volume and experience. Some studies included in this review found that long distance runners have lower bone mineral density compared to their non-running peers, but some report opposite results. McCormack et al (2019) found that male cross-country runners have greater bone mineral density at the femoral neck, total hip, and whole body than male non-running controls. Ravnholt et al (2018) found that intense intermittent running with 5 s sprints performed 3 days/week leads to lower body fat mass, as well as higher lean body mass and bone mineral density after only 7 weeks of training. They found an increase in plasma bone remodeling markers. We found no studies exploring increment interval training, which is a good way to start regular exercise for the sedentary population. Since it involves an increment in load, dropout rate is smaller than for high-intensity workouts.

Thus, the main goals of this study were: exploring how a short (8 weeks) interval aerobic exercise training program with increasing intensity affects the bone remodeling process in young untrained men and women and establishing if there was a connection between bone remodeling and body composition. The third goal was to determine if there were any sex differences in the effect of training on bone metabolism.

Based on the results presented in available literature, the main hypothesis of the present study is that there is a stimulating effect of the running program on the bone remodeling process (coupled and synchronized process of bone activation, resorption, reversal and formation), which would be demonstrated by changes in the concentration of markers of bone metabolism.

Materials and methods

General study design

Twenty-one college students voluntarily participated in the study. The inclusion criteria were: age between 20 and 30, non-athlete persons of both sexes. The exclusion criteria were any diagnosed bone disease or chronic illness that could influence bone metabolism, the use of diuretics or antacids, smoking, and any mineral/vitamin supplements taken during the study, which includes multivitamins and vitamin D supplementation in any form. Only women with a regular menstrual cycle were included in the study. The additional exclusion criteria for women was the use of hormone replacement therapy or contraceptives. All participants have completed the exercise protocol and finished the study. Every participant gave its written informed consent for participation in this study. The study protocol and procedures conformed to the standards set by the latest revision of the *Declaration of Helsinki* and the national legislation. The Ethical Committee of the University of Osijek, Faculty of Medicine approved the study (Approval number 2158–61-07-12-42).

Study protocol

This was a single group intervention. The measurements considering bone metabolism were taken two times: At the beginning of the study—before the start of the exercise program, and at the end of the study—after 8 weeks of exercises. The protocol of exercises, as well as the time points of sampling and measurements, are depicted in Fig. 1.

Measurements of anthropomorphic characteristics

Morphological characteristics were measured 6 times: 3 days before the start of the exercise protocol, after every 6th session, and at the end of the study (7 days after the last session). Weight and height of all participants were measured. The analysis of body composition was done with the use of the bioelectric impedance analysis (BIA) method, using the body composition analyzer GAIA 359 (Jawon Medical, Korea). All participants were informed about the requirements they had to fulfill before the body composition measurement: No food or drinks 4 h prior to the measurements, no exercise 12 h before the measurements, no alcohol consumption within 48 h before the measurements, and emptying the bladder 30 min prior to the measurement. The measurement results included: the body mass index (BMI), lean body mass (LBM), mass of body fat (MBF), percent of body fat (PBF), total body water (TBW), and mineral content (MIN). All measurements were performed in the morning (at 7–9 AM), by the same trained technician. The subjects were positioned to stand barefoot on two electrodes which were built in the pedestal of the instrument and hold another two electrodes in their hands, while electrical current was conducted. Body composition was calculated based on the amount of impedance by the manufacturer's software. The validity and reproducibility of GAIA 359 Plus has been reported by Jonker (2009), who obtained the correlation for the whole body FFM (fat free mass), measured by BIA and DXA $r = 0.96$, and that differences for %FFM and PBF (percent body fat) between BIA and DXA are -0.04% and 0.25%, respectively. The precision and repeatability of the BIA was determined by measuring 10 subjects during 4 consecutive days. %CV and ICC were calculated from these 4 sets of results. We obtained overall results as follows: for MBF: ICC = 0.946, CV = 1.9%, for PBF: CV = 1.93%, ICC = 0.941, for LBM: ICC = 0.742, CV = 1.7%, for MIN: ICC = 0.998, CV = 1.2%, for TBW ICC = 0.743, CV = 1%, for BMI: ICC = 0.895, CV = 1.1%.
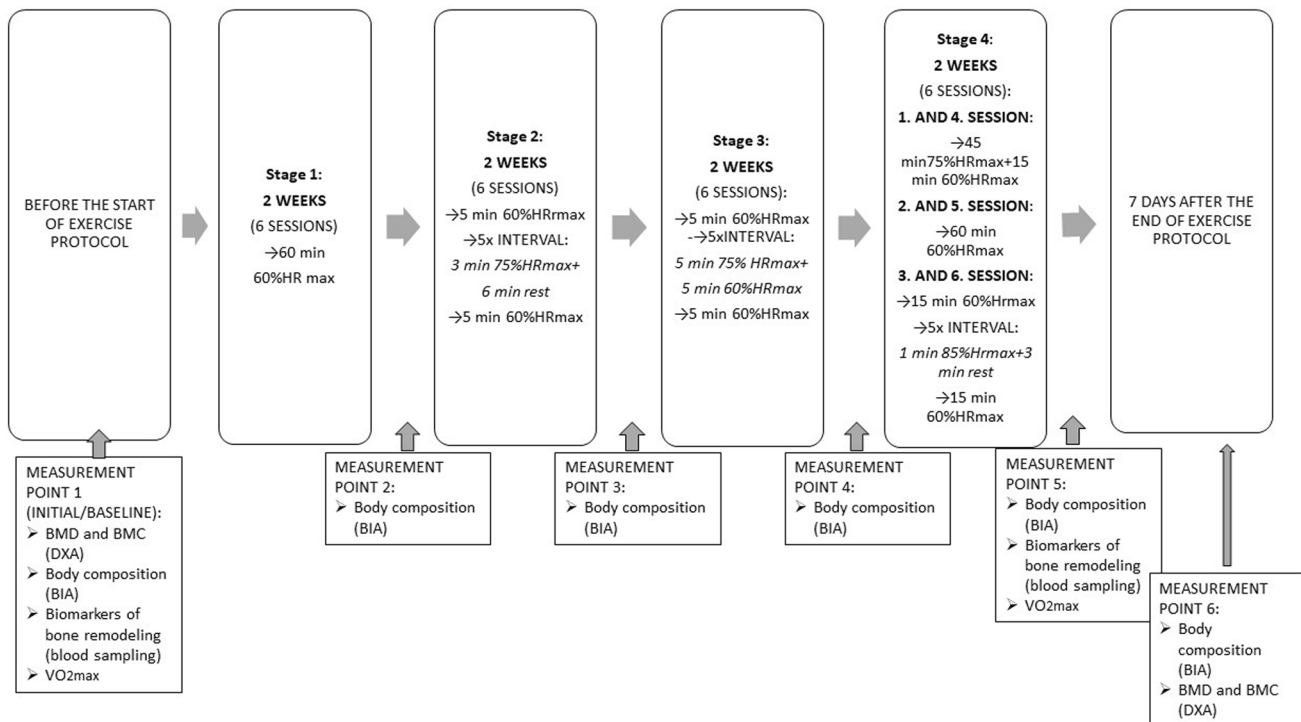
Fig. 1 Scheme of the study timeline—exercise protocol and measurement points

Bone density assessment

Bone mass density (BMD) and bone mineral content (BMC) were measured by dual x-ray absorptiometry (DXA) at three sites: at the lumbar spine L1–L4, and at the hips, dual femur (reported as the total mean of both femurs) and femoral neck (reported as the mean of the left and right femoral neck). The measurements were performed in the morning hours (between 7 and 9 am) on two occasions. First (baseline measurement): 3 days before the start of exercise training, and second: On the 7th day after the last training session. A trained technician using Lunar Prodigy 64575 G.E.S. S.A. took the measurements. The technician was blinded to other parameters obtained in the study. It has previously been reported that the precision error of the DXA scanner is between 0.6 and 1.8% (Shepherd et al. 2006).

Bone remodeling markers measurements

Blood samples were taken in the morning hours (between 7 and 9 am), after an overnight fast, on two occasions. At the start of the study: 1 day before the start of the exercise protocol, and at the end of the study: In the morning after the last exercise session (next day). The blood samples were used for the analysis of the bone remodeling biomarkers: osteocalcin (OC), C-terminal procollagen type I peptide (PICP), pyridinoline (PYD), parathyroide hormone (PTH), osteoprotegerin

(OPG), and the receptor activator of nuclear kappa B ligand (RANKL). The serum was removed after centrifugation at 1500 g for 10 min and stored at $-80°C$ before the analysis. The OC concentration was assessed using ELISA (Elabscience Biotechnology Co, Beiging, NR China), CV < 10%. The concentration of PICP was measured by ELISA (USCN Life Science Inc, Hubei, NR China), intra-assay CV < 10%, inter-assay CV < 12%. The PYD concentration was measured by ELISA (Qayee-Bio, Shangai, NR China), CV < 15%. The concentration of RANKL was measured by ELISA (Cusabio Biotech, Hubei, NR China), intra-assay CV < 8%, inter-assay CV < 10%.

The PTH (intact form) concentration was measured by ELISA (Calbiotech, Spring Valley, CA) with intra-assay CV < 10%, inter-assay CV < 12%. The OPG concentration was determined by ELISA (Kamiya Biomedical Company, Seattle, WA, SAD) with intra-assay CV < 12%, inter-assay CV < 14%.

Exercise protocol

The subjects practiced the exercise program for 8 weeks, in 1-h sessions, 3 times per week under supervision at the same time of day. The aerobic exercise consisted of running outside on an asphalt road. The maximum heart rate was calculated according to Tanaka et al (2000) as $HR_{max} = 208 - 0.7 \times$ years of age. For the first 2 weeks (6

Table 1 Median change in bone density and bone mineral content, and bone metabolism biomarkers with 95% confidence intervals for median change and effect size

	Median change Pre-post treatment (95% CI lower/upper)	P Wilcoxon test	d_{cohen}
BMD N.M. (g/cm^3)			
Whole sample	0.009 (− 0.002/0.02)	0.121	0.718
Men (N = 11)	− 0.011 (− 0.042/0.006)	0.824	0.096
Women (N = 10)	0.007 (− 0.040/0.012)	0.028*	1.088
BMD DF total (g/cm^3)			
Whole sample	0.002 (− 0.002/0.011)	0.259	0.507
Men (N = 11)	0.002 (− 0.005/0.030)	0.507	0.292
Women (N = 10)	0.004 (− 0.007/0.017)	0.374	0.391
BMD L1–L4 (g/cm^3)			
Whole sample	− 0.01 (− 0.031/0.004)	0.170	0.628
Men (N = 11)	− 0.011 (− 0.042/0.006)	0.155	0.653
Women (N = 10)	− 0.007 (−0.040/0.012)	0.646	0.201
BMC N.M. (g)			
Whole sample	− 0.03 (− 0.095/0.050)	0.509	0.291
Men (N = 11)	-0.035 (− 0.195/0.085)	0.594	0.234
Women (N = 10)	− 0.025 (− 0.110/0.170)	0.646	0.201
BMC DF total (g)			
Whole sample	0.210 (− 0.075/0.535)	0.145	0.670
Men (N = 11)	0.105 (− 0.43/1.415)	0.722	0.156
Women (N = 10)	0.325 (0.08/0.620)	0.028*	1.167
BMC L1–L4 (g)			
Whole sample	− 0.89 (− 2.140/0.275)	0.181	0.61
Men (N = 11)	− 0.965 (− 2.725/0.540)	0.155	0.635
Women (N = 10)	− 0.02 (− 4.04/0.7)	0.878	0.066
OC (ng/ml)			
Whole sample	6.320 (3.756/9.449)	0.001*	2.241
Men (N = 11)	6.315 (2.322/14.157)	0.016*	1.23
Women (N = 10)	5.930 (1.81/10.294)	0.022*	1.15
OPG (ng/ml)			
Whole sample	0.018 (− 0.202/1.786)	0.970	0.016
Men (N = 11)	0.014 (− 0.39/13.116)	0.959	0.224

Table 1 (continued)

	Median change Pre-post treatment (95% CI lower/upper)	P Wilcoxon test	d_{cohen}
Women (N = 10)	0.037 (− 0.214/2.473)	0.959	0.224
PICP (ng/ml)			
Whole sample	12.623 (− 0.195/24.896)	0.054	0.929
Men (N = 11)	11.947 (− 12.8/46.15)	0.286	0.478
Women (N = 10)	14.859 (− 18.41/26.60)	0.114	0.734
PTH (pg/l)			
Whole sample	− 3.915 (− 9.56/2.385)	0.279	0.485
Men (N = 11)	− 4.782 (− 10.110/1.735)	0.169	1.433
Women (N = 10)	− 3.585 (− 16.955/10.105)	0.721	1.299
PYD (nmol/l)			
Whole sample	5.54 (2.407/8.368)	< 0.001*	2.59
Men (N = 11)	3.818 (0.941/5.544)	0.008*	1.667
Women (N = 10)	8.265 (3.035/14.226)	0.013*	0.734
Rankl (pg/ml)			
Whole sample	87.75 (21.06/178.79)	0.001*	1.927
Men (N = 11)	92.256 (32.663/402.315)	0.003*	
Women (N = 10)	67.125 (− 12.322/271.144)	0.114	

BMD bone mineral density, *BMC* bone mineral content, *N.M.* neck mean (femoral neck (reported as mean of left and right femoral neck)), *DF TOTAL* dual femur (reported as total mean of both femurs), *L1-L4* lumbar spine L1-L4, *OC* osteocalcin, *OPG* osteoprotegerin, *PICP* C-terminal procollagen type I peptide, *PTH* parathyroid hormone, *PYD* pyridinoline, *RANKL* receptor activator of nuclear kappa B ligand

p < 0.05

*Statistically significant change in variable following exercise for 8 weeks

sessions) a low intensity training (60% HR_{max}) during 45–60 min was conducted. The next 2 weeks (6 sessions) consisted of sessions with changing intensity: A 3 min working interval with 75% HR_{max} was followed by a 6 min rest. A session started with running at 60% HR_{max} during 5 min, followed by interchanging intervals (total of 5 intervals per session). The next 6 sessions consisted of 5 min working intervals (75% HR_{max}), followed by a 5 min low intensity interval (60% HR_{max}) (the total of 5 interval changes per session). The last 2 weeks (6 sessions) were performed in a way that every first session consisted of working intervals with the intensity of 75% HR_{max} followed by a rest period; every second session had working intervals with lower intensity of 60% HR_{max} followed by a rest period, and every third session had working intervals loaded to 80–85% HR_{max} followed by

a rest period. The sessions with maximum load started with 15 min of running at 60% HR_{max}, followed by 5 intervals of interchanging load, with a 1-min working interval and a 3-min rest. After the completion of the intervals, the running session ended with 15 min of running at 60% HR_{max}. Every running session ended with a stretching exercise. To evaluate maximum oxygen uptake, the Cooper test (Cooper 1968) was performed before the start and at the end of the exercise program (next day after the last exercise session). The Cooper test was performed by running on a treadmill (Precor 966i, USA) for 12 min, and VO2max was calculated from the distance covered by running in this period of time (Tanaka et al 2000). According to the calculated VO2max expressed as ml/kg/min, the subjects were assigned to one of 5 categories of physical fitness (Strasser 2018):

very poor (men < 33, women < 23.6), poor (men 33–36.5; women 23.6–28.9), fair (men 36.5–42.4; women 29.0–32.9), good (men 42.5–46.4; women 33.0–36.9), excellent (men 46.5–52.4; women 37.0–41.0), and superior (men > 52.4; women > 41.0), so that the exercise protocol could be adjusted for every physical fitness category and performed separately for each category. The exercise sessions were practiced for each category separately, with adjusted intensity according to the physical fitness of the group. The exercise intensity was monitored by heart rate monitors Polar RS400. Engstrom et al. (2012) reported the validity and repeatability of the Polar RS400 in comparison with ECG, and they found no differences between the results for the two methods (mean difference of 0.7 bpm). The difference between the two measurements was the mean of 2.6 bpm.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 20. The results are presented as the arithmetic mean $\pm$ SD. Because the sample size was < 30, non-parametric tests were used. The differences between the initial values and the final values of variables describing bone metabolism (DXA measures and biomarkers of bone metabolism) and VO2max were tested by the Wilcoxon Signed Rank test (for the whole sample and separately for each sex), while the Friedman test with the Bonferroni corrected post hoc tests were used for variables describing body composition (BIA measures) because they were measured on 6 occasions during the study (for the whole sample and separately for each sex). The differences between the sexes at the start of the study and at the end of the study were tested by the Mann Whitney U test. 95% CI for median difference between the sexes or between the initial and final measurements was determined by the appropriate Hodges Lehman estimation (for the difference between sexes, the HL estimation for independent samples, and for the change between pre and post-treatment values, the HL estimation for dependent samples). The effect sizes are calculated based on statistical tests results with the help of an online calculator (Lenhard and Lenhard 2016). The level of statistical significance was set at $p = 0.05$.

Results

The sample consisted of 11 men and 10 women, with the mean age of 22 (from 20 to 23). The measurement of physical fitness (VO$_{2max}$) confirmed their improvement in fitness. The VO$_{2max}$ value increased from 35.47 ± 10.8 ml/kg/min at the start of the study to 42.18 ± 9.7 ml/kg/min at the end ($p < 0.0001$). Men had a better score of VO$_{2max}$ at the start of the study (mean value $\pm$ SD $= 42.5 \pm 8.9$ ml/kg/min)

compared to women (mean value $= 27.7 \pm 6.8$ ml/kg/min). However, the exercise regime caused a smaller improvement of VO$_{2max}$ in men (mean change $= 5.8$ ml/kg/min (13.6%), $p < 0.0001$) than in women (mean change $= 7.7$ ml/kg/min (28%), $p = 0.0002$).

The effect of exercise on bone remodeling and body composition

Pre- and post- intervention results and median differences and 95% CI for median difference for the bone remodeling markers and DXA results are presented in Table 1. The results are presented graphically in Figs. 2 and 3. There were no statistically significant changes in bone density and bone mineral content at the measured sites when tested for the whole sample. However, we found a statistically significant increase in the BMD neck mean in women, while there was no statistically significant change in men and in the whole sample. There was also a significant increase in dual femur total BMC in women, while no statistically significant change was observed in men and in the whole sample. Effect sizes (reported in Table 1 as d_{Cohen}) indicate large or intermediate effects of the exercise program on the change in bone density and bone mineral content at measured sites. The concentration of some biochemical markers of bone metabolism have also changed significantly. The concentration of pyridinoline (PYD), a marker of bone resorption and the concentration of osteocalcine (OC), a marker of bone remodeling in general, have been significantly increased after 8 weeks of the exercise program compared to their concentrations before the exercises (the baseline value). The concentration of the receptor activator of nuclear kappa B ligand (RANKL) was also significantly increased after the exercise program, compared to the start (baseline) value. All of these changes are observed in the whole sample and separately in both sexes, except for the increase in RANKL, which was not statistically significant in women.

Among the body composition variables (the change of body composition during the study is presented in Table 2, the exercise protocol affected only the mass of body fat (MBF) and the percent of body fat (PBF) significantly. The time course of the changes in the results of the body composition assessment during the study are presented in Fig. 4. Figure 4A represents individual changes in MBF and PBF for each subject, while the boxplots in Fig. 4B represent median and interquartile range overlaid with individual results. MBF was declining as the intensity of exercises increased, but after completing the exercise program, when subjects stopped exercising, it increased. The same was with PBF. Pairwise comparisons showed statistically significant differences between point of measurement 2 and points of measurement 4 and 5 for both variables (Fig. 4A,B). The separate analysis for sexes revealed that the changes in MBF
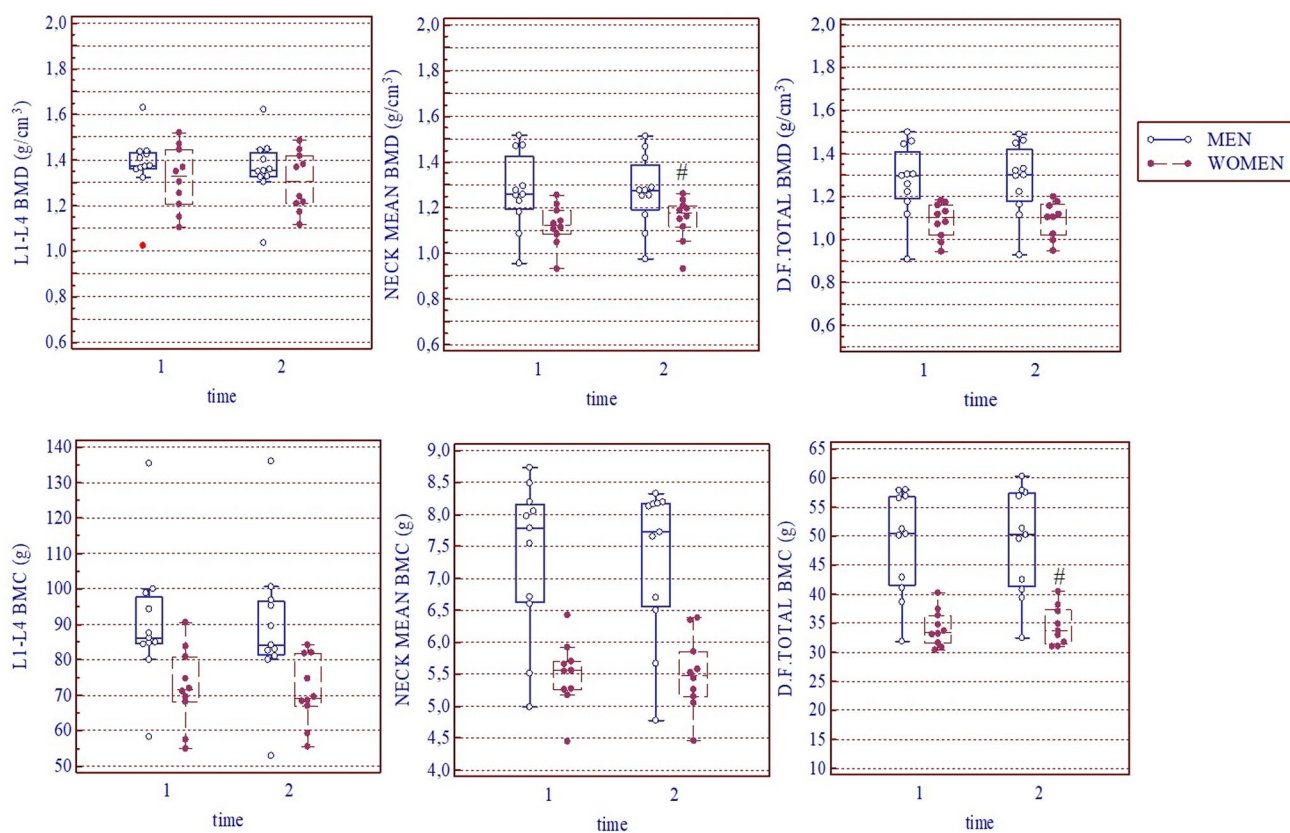
Fig. 2 Results of the DXA measurements pre-treatment (1) and post-treatment (2) for men and women. $p < 0.05$ # statistically significant change from the pretreatment value

and PBF during the study were statistically significant only in men, and not in women (Table 2).

Sex-related differences in bone metabolism and body composition

Results of bone density, bone mineral content, bone metabolism biomarkers, body composition, and differences between men and women, at the start and at the end of the study, with 95% CI median difference and effect sizes are presented in Table 3. BMD and BMC in most of the measured sites were significantly higher in men compared to women, and these differences were not altered by the exercise regime. The concentration of biochemical markers of bone remodeling were not different between the sexes at the start or at the end of the study. Men had significantly higher values of lean body mass (LBM), total body water (TBW), and body mineral content (MIN), and lower values of the percentage of body fat (PBF) compared to women, while there was no statistically significant difference between sexes for the mass of body fat (MBF) or the body mass index (BMI). The aerobic training program which was performed for 8 weeks did not affect these differences between sexes.

Discussion

The most important finding in the present study is that the applied increment interval aerobic exercise program in the duration of 8 weeks was accompanied by the increase in concentration of biochemical markers of bone metabolism (PYD, OC, and RANKL) at the end of the exercise program, and the increase in BMD neck mean BMD and BMC dual femur total in women, but not in men.

In the present study, the concentration of PYD increased, suggesting increased bone resorption (Banfi et al 2010). PYD is not the preferred biomarker of bone degradation, as it is a very small molecule that is quickly lost in urine, but we believe that the eventual loss of PYD in urine would probably be similar in both occasions of blood sampling (at the start and at the end of the study), since the same persons were tested under the same conditions at both occasions. Nevertheless, PYD was increased after the training period, and the magnitude of the increase of the PYD concentration at the end of the exercise program was more pronounced in women than in men (median increase 8.26 nmol/l vs 3.82 nmol/l). The possible explanation for this observation was that estrogen might influence the mechanism of PYD release from the collagen in the bone matrix during
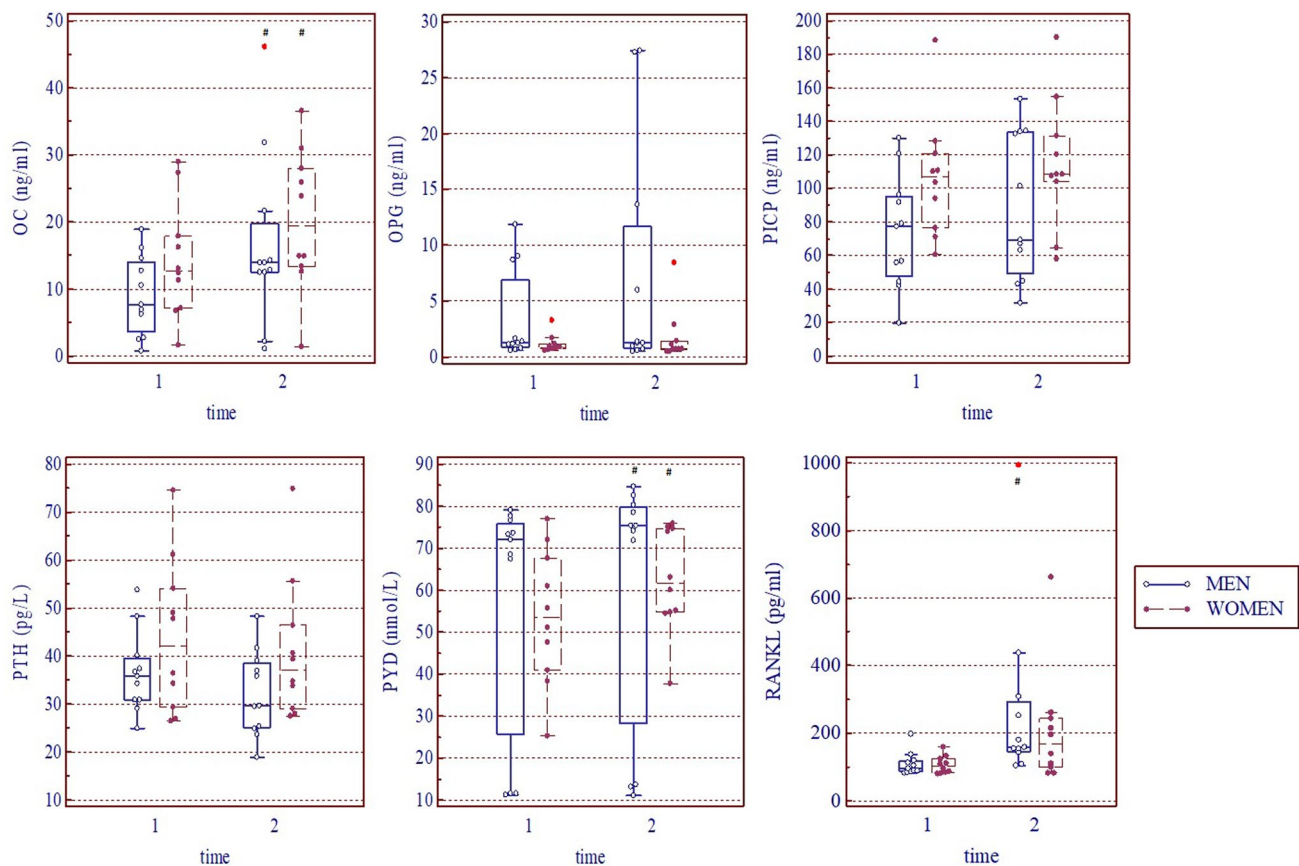
Fig. 3 Results of the serum concentration of bone metabolism biomarkers at the start (pretreatment—labeled as 1) and at the end (post-treatment—labeled as 2) for men and women. $p < 0.05$ # statistically significant change from the pretreatment value

bone resorption. However, this remains to be supported by experimental measurements. Furthermore, the concentration of OC increased, suggesting an increased activity of osteoblasts (Banfi et al 2010), and an increase in the bone remodeling process. Although there is evidence from literature (Weaver et al 1997) that the serum level of OC is highly correlated to bone formation measured by calcium kinetics (Vo+) ($r = 0.82$, $p = 0.001$), it is irrefutable that OC can be released during bone resorption, which suggests that this biomarker indicates bone remodeling in general rather than bone forming. The biomarker of bone formation PICP did not change significantly in this study. PICP originates, the same as PINP, mostly from proliferating osteoblasts. PINP is the preferred marker for bone formation, because PICP is cleared by the mannose receptor, which can be regulated by the growth hormone and thyroid hormones, so its interpretation could be complicated in patients with thyroid dysfunction (Shetty et al 2016). Our subjects were healthy young people, and we did not expect this confounding to happen. Although we did not find a statistically significant change in PICP, d_{Cohen} (Table 1) indicates an intermediate or large effect size, which means that we cannot disregard

the possibility that there could be an effect of the exercise program on the PICP concentration. It seems that this training program, which consisted of interval aerobic exercise with increment intensity, was able to override the effect of everyday physical activity, since we found an increase in markers of bone remodeling, and most of the markers had intermediate or large effect size, which indicates the possibility of change that our study did not observe. These results are different from reported results of Woitge et al (1998), who found no difference in bone degradation markers after 8 weeks of the aerobic exercise program. The present study had a different exercise program, we included increment interval training which could affect bone metabolism through different mechanisms compared to a continuous exercise load and unchanged exercise intensity. It has been shown that intermittent exercise programs are more effective in the acceleration of bone turnover (Robling et al 2001; Ravnholt et al 2018) and the increase of bone formation, since osteocyte desensitization is avoided with the intermittent load. Additionally, the increase in the concentration of RANKL, the biochemical marker of bone remodeling, confirms the enhancement of the bone remodeling process due

Table 2 Results for body composition during the study presented as median (interquartile range IQR) with the results of the Friedman test for the significance of change and the effect size

	Point of measurement						Friedman test p	Kendalls W
	1 Median (IQR)	2 Median (IQR)	3 Median (IQR)	4 Median (IQR)	5 Median (IQR)	6 Median (IQR)		
PBF (%)								
Whole sample	24.65 (18.4–29)	24.85 (19–29.35)	24.1 (18.3–28.8)	23.05 (17.45–28.15)	23.9 (17.05–27.8)	24.15 (18.2–28.2)	<0.001*	0.246
Men ($N = 11$)	19 (17.2–26.8)	19.3 (16.9–25.9)	18.6 (17.5–25.0)	17.9 (16–25.4)	17.2 (16.7–25.3)	18.7 (17.8–26.7)	0.005*	0,302
Women ($N = 10$)	28.0 (24.85–30.95)	28.9 (24.65–30.75)	28.2 (24.1–30.25)	28.0 (23.05–30)	27.8 (23.9–30.25)	27.3 (24.1–30.8)	0.124	0,192
MBF (kg)								
Whole sample	16.9 (13.4–21.75)	17.2 (13.78–21.75)	16.35 (14–21.05)	16.5 (12.3–20.65)	16.45 (13.1–20.4)	17.1 (13.5–21.4)	<0.001*	0.264
Men ($N = 11$)	15.8 (11.7–22)	15.9 (12.6–21.8)	15.5 (12.9–21.1)	14.3 (12–20)	14.1 (11.7–20.4)	15 (11.9–23.1)	0.003*	0,323
Women ($N = 10$)	19 (16–21.55)	19.1 (14.95–21.9)	18.7 (14.5–21.2)	18 (14.6–20.75)	17.9 (14.75–20.9)	17.9 (14.7–21.3)	0.128	0,190
LBM (kg)								
Whole sample	56.3 (48.1–67.4)	57.5 (48–67.9)	57.7 (48.3–67.5)	58.7 (48.6–67.5)	60.1 (47.9–67.5)	57.8 (48.1–69.5)	0.159	0.084
Men ($N = 11$)	66.8 (62–72.7)	66.3 (62.1–69.8)	66.3 (61.1–70.8)	66.9 (61.6–71.9)	65.7 (61.2–68.8)	65.3 (61.3–71.4)	0.521	0,076
Women ($N = 10$)	47.85 (45.9–50.1)	47.9 (44.9–49.325)	48 (44.8–50.5)	48.15 (45.15–49.9)	47.65 (44.87–49.2)	47.75 (47.7–49.1)	0.235	0,170
TBW (kg)								
Whole sample	40.5 (34.6–48.5)	41.4 (34.6–48.9)	41.5 (34.8–48.6)	42.3 (35–48.6)	43.3 (34.5–48.6)	41.6 (346–50)	0.198	0.077
Men ($N = 11$)	48.1 (44.6–52.3)	47.7 (44.7–50.3)	47.7 (44–51)	48.2 (44.4–51.8)	47.3 (44.1–49.5)	47 (44.1–51.4)	0.539	0,074
Women ($N = 10$)	34.45 (33–36.05)	34.5 (32.3–35.5)	34.55 (32.3–36.35)	34.65 (32.55–36)	34.3 (32.32–35.45)	34.35 (32.1–35.3)	0.299	0,152
BMI (kg/m^2)								
Whole sample	23.9 (21.9–25.7)	24 (22.4–25.5)	23.9 (22.2–25.5)	23.9 (22.1–25.5)	23.5 (21.8–25.4)	23.7 (22.4–26.1)	0.056	0.131
Men ($N = 11$)	24.3 (22.9–27.4)	24.5 (22.8–27)	24 (22.9–27.3)	23.9 (22.4–27)	24 (22.4–26.6)	23.9 (22.8–27)	0.106	0,165
Women ($N = 10$)	23.35 (21.6–25.1)	23.5 (21.12–24.95)	23.35 (21.3–24.9)	23.15 (21.17–24.8)	23.05 (21.2–24.9)	22.8 (21.5–25.1)	0.381	0,132
MIN (kg)								
Whole sample	4.4 (4.5–5.1)	4.4 (3.9–4.8)	4.4 (3.9–4.8)	4.2 (3.9–4.7)	4.3 (3.8–4.7)	4.4 (3.9–4.9)	0.05	0.117
Men ($N = 11$)	4.7 (4.5–5.1)	4.7 (4.5–4.9)	4.7 (4.5–5.0)	4.7 (4.5–4.9)	4.7 (4.5–4.9)	4.8 (4.4–5.0)	0.234	0,124
Women ($N = 10$)	3.9 (3.65–3.97)	3.9 (3.5–3.9)	3.9 (3.47–4.0)	3.9 (3.37–3.9)	3.85 (3.37–3.9)	3.9 (3.45–4.1)	0.327	0,145

$p < 0.05$, *statistically significant change in variable following exercise for 8 weeks

Abbreviations: BMI *body mass index*, LBM *lean body mass*, MBF *mass of body fat*, PBF *percent of body fat*, TBW *total body water*, MIN *mineral content of the body*

to the increment intensity of the training protocol. It has been shown that RANKL is involved in the response of the bone to a mechanical load via the increase in differentiation and the activity of osteoclasts, bone degradation cells (Ikeda et al 2004). It is obvious from our results that the range of results for the serum RANKL was much wider at the end than at the start of the study, which could be confounding for result interpretation and reliability. Another stimulus for
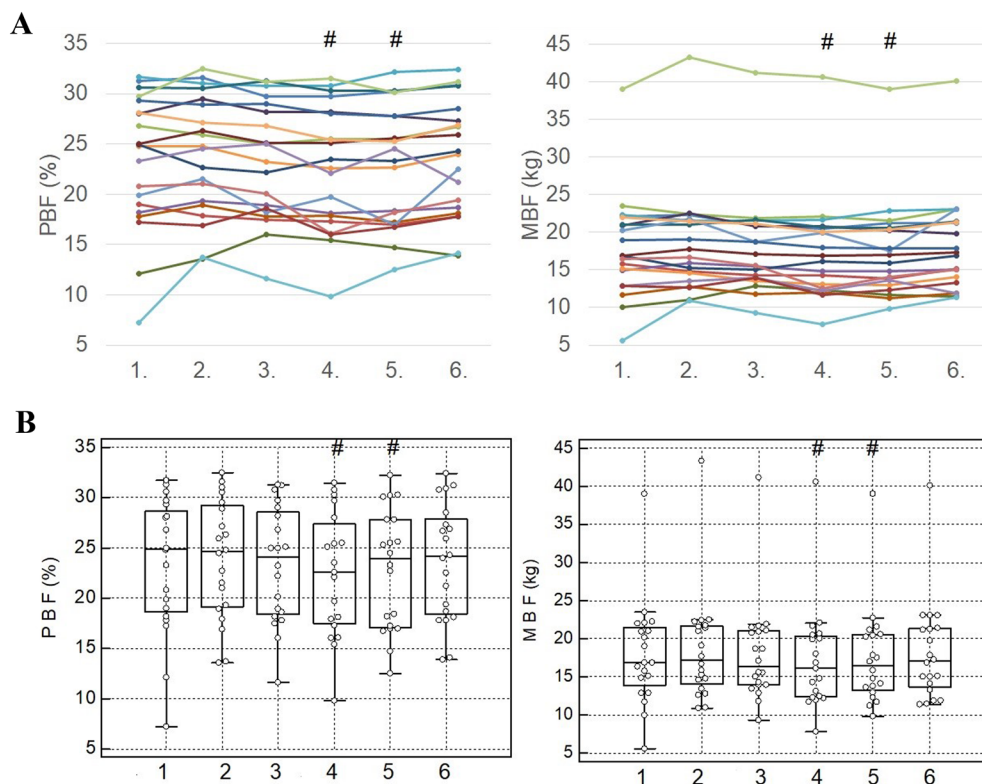
Fig. 4 Results of body composition assessments for MBF (mass of body fat) and PBF (percent of body fat) during the study (6 measurement points labeled with numbers 1 to 6) $p < 0.05$ # statistically significant change from the point of measurement 2. **A** Individual changes of each subject during the study. **B** The boxplots overlaid with individual data points during the study

the increase in RANKL expression is PTH (Bouassida et al 2006). However, PTH concentration was not increased significantly in the present study (Table 1), possibly because the mechanical load of the exercise used in the present study was not strong enough to increase PTH production. This result is in agreement with a similar study by Bouassida et al (2006). Also, the effect size for PTH could indicate that it is possible that PTH changed even though we did not get a statistically significant change, but the direction of this change was towards lower concentration, which is definitely not the result we expected for exercise induced remodeling with PTH involvement. Alternatively, some other mechanism for the increase in RANKL might be involved, or it is possible that the elevated concentration of PTH returned to its baseline value before the collection of blood samples, as it was previously described (Thorsen et al 1997). Interestingly, the literature data on the influence of sex differences on the dynamics of markers of bone metabolism is scarce. For example, some differences in the response to extreme loads could be explained by the role of estrogen in the remodeling process (Joseph et al 2005). In the present study, we found somewhat different results between the sexes when we tested men and women separately for pre-post differences. The increase in RANKL was statistically

significant for men, while it was not statistically significant for women. It has been reported that sex hormones influence the mechanisms of bone remodeling and the role of RANKL, by altering its expression (Streicher et al 2017), and our study confirmed different effects in men vs. women regarding exercise induced remodeling. (Fig. 2).

A typical bone remodeling cycle occurs over 120–200 days. It consists of several stages: resorption (2-week duration), reversal (4–5-week duration), formation (up to 4-month duration) and termination at the end of this cycle (Kenkre and Bassett, 2018), with the optimal length of three months for DXA to detect the changes in mineralization. However, the changes in bone density after 9 weeks and even after 7 weeks of exercising were described in literature (McWhannell et al 2008; Ravnholt et al 2018). In the present study, a separate analysis of changes pre-post treatment showed different effects of training for men and women. While there was no change in any of the DXA measurements for men, we found an increase in the femoral neck mean BMD and an increase in BMC dual femur total in women. These changes were small (95%CI median change for femoral neck mean BMD from 0.004 to 0.056 g/cm³, and 95% CI median change for dual femur total BMC from 0.08 to 0.620 g) but statistically significant. These results

Table 3 Median differences between the sexes at the start (pre-treatment) and at the end (post-treatment) of the study, along with the 95% confidence intervals, statistical significance, and effect size

	Pre-treatment					Post-treatment				
	Women (N=10) Median (IQR)	Men (N=11) Median (IQR)	Median Difference (95% CI lower/upper)	p	d_Cohen	Women (N=10) Median (IQR)	Men (N=11) Median (IQR)	Median difference (95%CI lower/upper)	p	d_Cohen
DXA measures										
BMD N.M. (G/CM³)	1.122 (1.07–1.19)	1.257 (1.18–1.47)	0.146 (0.039/0.297)	0.014*	1.276	1.176 (1.1–1.21)	1.277 (1.17–1.4)	0.102 (0.017/0.203)	0.029*	1.084
BMD DF TOTAL (G/CM³)	1.10 (1.01–1.16)	1.29 (1.17–1.45)	0.184 (0.092/0.315)	0.005*	1.528	1.103 (1.01–1.16)	1.3 (1.16–1.45)	0.197 (0.063/0.332)	0.009*	1.558
BMD L1–L4 (G/CM³)	1.328 (1.19–1.45)	1.37 (1.36–1.43)	0.69 (−0.73/0.204)	0.324	0.44	1.304 (1.2–1.4)	1.354 (1.33–1.44)	0.078 (−0.066/0.188)	0.526	0.279
BMC N.M. (G)	5.55 (5.24–5.7)	7.79 (6.6–8.2)	2.19 (1.05/2.79)	0.005*	1.558	5.48 (5.13–5.98)	7.72 (6.51–8.18)	2.06 (1.07/2.72)	0.002*	1.761
BMC DF TOTAL (G)	33.48 (31.41–36.6)	50.34 (41.0–56.9)	16.61 (7.81/21.8)	0.001*	2.088	33.7 (31.36–37.64)	50.24 (40.87–57.54)	16.42 (7.62/22.62)	0.001*	2.185
BMC L1–L4 (G)	71.53 (65.5–81.6)	86.21 (84.4–98.9)	15.49 (5.34/28.76)	0.006*	1.497	69.14 (65.1–81.8)	84.15 (81.1–96.89)	15.29 (6.32/27.49)	0.011*	1.328
Biochemical markers of bone remodeling										
OC (NG/ML)	12.77 (7.07–20.3)	7.65 (2.8–14.6)	−4.69 (−11.65/1.45)	0.181	0.611	19.4 (13.2–28.7)	13.9 (12.5–21.7)	−2.43 (−14.05/5.89)	0.231	0.541
OPG (NG/ML)	0.887 (0.72–1.29)	1.266 (0.76–8.71)	0.38 (−0.157.02)	0.193	0.593	0.73 (0.64–1.8)	1.266 (0.74–13.7)	0.527 (−0.146/12.25)	0.170	0.628
PICP (NG/ML)	107.05 (75.1–122.7)	77.21 (44.4–96.4)	−31.5 (−64.3/1.8)	0.072	0.852	108.5 (94.8–137.3)	69.34 (44.68–134.2)	−31.42 (−64.38/21.99)	0.260	0.507
PTH (PG/L)	42.15 (28.72–55.9)	35.85 (30.8–40.19)	−5.43 (−19.79/4.78)	0.418	0.359	37.04 (28.8–48.8)	29.76 (24.9–39.1)	−5.33 (−16.74/1.96)	0.181	0.611
PYD (NMOL/L)	53.49 (40.3–68.8)	72.113 (11.6–76.1)	10.5 (−14.02/26.9)	0.275	0.49	61.65 (54.7–74.8)	75.46 (13.7–80.3)	7.01 (−26.78/20.29)	0.260	0.507
RANKL (PG/ML)	101.65 (84.5–126.3)	95.12 (87.6–120.5)	1.32 (−22.4/22.4)	0.778	0.123	167.31 (96.4–248.4)	158.15 (142.9–308.4)	34.26 (−71.92/140.45)	0.398	0.375
Measures of body composition										
BMI (KG/M²)	23.35 (21.6–25.1)	24.3 (22.9–27.4)	−1.4 (−4.4/0.9)	0.152	0.430	22.8 (21.5–25.1)	23.9 (22.8–27)	−1.5 (−4.7/0.3)	0.095	0.541
LBM (KG)	47.85 (45.9–50.1)	66.8 (62–72.7)	−18.7 (−26.7/−11.8)	<0.001	1.835	47.75 (47.7–49.1)	65.3 (61.3–71.4)	−17.70 (−25/12.70)	<0.001	1.835
MBF (KG)	19 (16–21.55)	15.8 (11.7–22)	2.1 (−3.0/7.3)	0.295	0.628	17.9 (14.7–21.3)	15 (11.9–23.1)	1.9 (−3.40/6.30)	0.503	0.457
PBF (KG)	28.0 (24.85–30.95)	19 (17.2–26.8)	7.6 (3.2/12.8)	0.007	1.558	27.3 (24.1–30.8)	18.7 (17.8–26.7)	7.10 (1.80/12.0)	0.012	1.439
TBW (KG)	34.45 (33–36.05)	48.1 (44.6–52.3)	−13.5 (−19.3/−8.5)	<0.001	1.835	34.35 (32.1–35.3)	47 (44.1–51.4)	−12.80 (−18.0/−9.10)	<0.001	1.835

Table 3 (continued)

	Pre-treatment					Post-treatment				
	Women (N=10) Median (IQR)	Men (N=11) Median (IQR)	Median Difference (95% CI lower/upper)	p	d_{Cohen}	Women (N=10) Median (IQR)	Men (N=11) Median (IQR)	Median difference (95%CI lower/upper)	p	d_{Cohen}
MIN (KG)	3.9 (3.65–3.97)	4.7 (4.5–5.1)	−0.9 (−1.5/−0.6)	<0.001	1.41	3.9 (3.45–4.1)	4.8 (4.4–5.0)	−0.9 (−1.5/−0.5)	<0.001	1.439

BMD bone mineral density, *BMC* bone mineral content, *N.M.* neck mean (femoral neck (reported as mean of left and right femoral neck)), *DF TOTAL* dual femur (reported as total mean of both femurs), *L1-L4* lumbar spine L1-L4, *OC* osteocalcin, *OPG* osteoprotegerin, *PICP* C-terminal procollagen type I peptide, *PTH* parathyroid hormone, *PYD* pyridinoline, *RANKL* receptor activator of nuclear kappa B ligand, *BMI* body mass index, *LBM* lean body mass, *MBF* mass of body fat, *PBF* percent of body fat, *TBW* total body water, *MIN* mineral content of the body

($p < 0.05$, *statistically significant differences between sexes)

indicate that the training program had different effect on the sexes. Men had higher bone density and bone mineral content compared to women at the start and at the end of the study. There is some presumption that higher bone density and bone mineralization is connected to higher LBM in men (Vicente-Rodrigez et al 2008), which is evident from the results presented in Table 3, and it is typically considered to be the result of differences in the hormonal status between sexes, but it could also not be disregarded that it could be correlated to physical activity during childhood and adolescence. As Ruiz et al (2011) showed, men are more active than women during adolescence. The higher VO_{2max} in men demonstrated the higher physical fitness level in men than in women at the beginning of the protocol. This could be a reason for the difference in the change of VO_{2max} between men and women after the exercise program in this study. After the study, VO_{2max} improved more for women than for men (delta (value before – value after the protocol) in VO_{2max} for women was on average 7.7 ml/kg/min vs. 5.8 ml/kg/min for men). The performed exercise program brought women from the poor VO_{2max} of 27.68 ml/kg/min to the fair VO_{2max} of 35.37 ml/kg/min, while the improvement for men was from the fair VO_{2max} of 42.55 ml/kg/min to the excellent VO_{2max} of 48.36 ml/kg/min. The difference in physical fitness between the sexes could be the confounding variable for interpreting differences found in other variables, and this can be considered as a limitation of the present study. The effect of the training program on bone metabolism and body composition was not an issue for the main outcome of the present study, since each subject was its own control, and results post-treatment were compared to the same subject pre-treatment.

Aerobic exercise can lead to the reduction of visceral fat which is not correlated to body weight reduction (Johnson et al 2009). In the present study, the only significant effect of the 8 weeks of aerobic exercise was on the mass of body fat (MBF), and consequently on the percent of body fat (PBF). Furthermore, the results demonstrated a decrease in MBF as the intensity of exercises was increasing, until the 4th measurement, while the MBF values were increasing in the last two measurements (Table 2, Fig. 4B). Our results comply with the results of previous studies (Coker et al 2009; Lee et al 2012) and show a negative relationship of aerobic exercise intensity and the mass of body fat. The increase in mass of body fat which was recorded after quitting the exercise protocol was similar to the results of Eastwood et al (2012). However, as King et al (2007) presumed, every individual person responds to the energy deficit produced by aerobic exercises in its own unique way. The difference in MBF between sexes is known and documented in literature (Duncan et al. 2004). Observed changes in body fat mass, although statistically significant, were in a very smallamount, in other words, the changes in MBF and PBF were

too small to be clinically important. The median difference between the highest and the lowest value for PBF (2nd and 4th measurement) was 1.1 (IQR 0.45–1.87), which is a decrease of 4.4% from the baseline value for PBF. The least significant change, calculated as 2.77*CV, would be 8.11%. The median decrease in MBF from point of measurement 2 median 17.2 kg to the point of measurement 4 median value 16.5 kg was 1.1 (IQR 0.35–1.77) kg or 6.4% of the baseline value. The least significant change of MBF would be 8%. -Effect sizes (Kendall's *W*) showed small effect or no effect at all.

There was no statistically significant change in the lean body mass (LBM) after the exercise program. That is in accordance with the results of some studies that demonstrated no effects of aerobic exercise to LBM in overweight persons (Park et al 2003), sedentary women (Antonio et al 2000), and children 11–13 years old (Dashti 2011).

The limitation of this study is that dietary habits of participants were not controlled or recorded. However, the participants were advised not to change dietary or any other lifestyle habits during the study period. We cannot exclude the possibility that the increase in energy expenditure increased their appetite and energy intake in the amount that could influence the results of body composition measurements. Even though the influence of the diet should not be neglected (Bopp et al 2008; Josse et al 2011), some researchers did not notice changes of LBM following aerobic exercise even with a controlled diet (Frykman et al 2003), similar to the results of the present study. Another limitation of the study is a lack of a non-trained control group. All subjects were measured twice: at the start of the study (pre-intervention) and at the end of the study (post-intervention). Pre-intervention measurements served as a control for a comparison with post-intervention measurements, for changes evoked by the study protocol. That way we avoided the biological diversity of the control and the experimental group and avoided the alteration in biomarkers not related to our study protocol. However, the comparison of each individual to their own baseline results does not allow consideration of typical errors or usual variations across the study time period. A control group would be useful for quantifying non-interventional sources of variation and as assistance in isolating results as being due to the exercise program performed in the study, so the lack of such a group is a limitation of this study.

In conclusion, the interval increment aerobic exercise protocol implemented for 8 weeks affected the bone remodeling process, which was evident from the increase in concentration of some bone remodeling biomarkers. The results also revealed somewhat different effects of the exercise program on the bone metabolism in men vs women involved in this study.

Declarations

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