# Bone mineral density and bone turnover in male masters athletes aged 40-64

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#### Abstract

We evaluated areal bone mineral density (aBMD), bone mineral content (BMC), and markers of bone turnover in male competitive masters athletes representing different training profile in the past and at present, aged 40–64 (14 endurance runners, and 12 speed-power athletes), and non-sport controls (n = 13). Dual-energy X-ray absorptiometry measurements of total body and regional aBMD, BMC and soft tissue composition were acquired. Serum concentrations of osteocalcin (OC), C-terminal crosslinking telopeptide of type I collagen (CTX), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), total testosterone (TT), free testosterone (FT) and insulin like growth factor-1 (IGF-1) were measured. Adjusted total and regional aBMD and BMC (covariates: body mass, body height and age) were significantly greater in all measured regions in speed-power athletes than in endurance athletes and control subjects, but adjusted aBMD and BMC values were not significantly different between endurance athletes and controls. No differences in bone formation (OC), bone resorption (CTX), and serum concentrations of TNF- $\alpha$ , TT, FT and IGF-1 were noted. This suggests that weight-bearing exercise in young age and the training continuation in later life may be an important contributor to the aBMD and BMC in the middle age and in the elderly. It seems also that training-related bone differences in men are not caused by present alterations in bone turn-over or somatotropic effects. However, conclusions must be drawn with caution due to a large variability of biochemical markers.

Keywords: Bone mass, bone metabolism, body composition, masters athletes

## Introduction

Physical activity is an important factor modifying bone tissue mass. It is well known that regular exercise enhances peak bone mass and preserves the agerelated decreases in bone mineral density (BMD) [1]. Some investigators have shown that athletes after many years of training or after the end of their sport career have higher areal bone mineral density (aBMD) in the most overloaded sites, depending on the trained kind of sport, in comparison with nonpracticing subjects [2]. Especially, weight-bearing exercises have osteogenic influence on bones [3].

In contrast with that, an excessive physical activity may have a negative effect on the skeleton, especially on sites containing a larger proportion of trabecular bone, which is sensitive to endocrine status [4,5]. Cross-sectional studies have revealed that athletes practicing endurance running have usually lower values of BMD in the lumbar spine and femoral neck in comparison with those practicing weight training, and some results show the same or lower values in comparison with nonpracticing subjects [6,7].

Vigorous exercise increases bone mass mainly in sites exposed to loading forces, but the physical activity also influences bone metabolism by modification of endocrine system [8]. It has been demonstrated that regular intensive training affects, among other things, the chronic increase in testosterone, dehydroepiandrosterone and insulin-like growth factor-1 (IGF-1) concentrations [9,10].

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However, the effect of exercise may depend on the stage of age [8]. Animal studies demonstrate that bone modelling does become less active in adult age than during growing and bones appear less responsive to the mechanical loading [11]. After the age of 40, physiological changes in bone may be intensified by hormonal factors [12]. There is a decrease of growth hormone secretion as well as diminished concentration of IGF-1 in serum [13]. Much evidence demonstrates gradual reduction of activity of interstitial cells (Leydig cells) during ageing. Among other things, a decreased concentration of total and free testosterone (FT) as well as an increase in concentration of sex hormone binding globulin in serum have been observed [14].

Bone density in adults is known to decline with age. Lanyon and Skerry [15] hypothesise that bone loss contributes to diminished loading-related stimulation resulting from a decline in both the absolute level of physical activity and its osteogenic potential. Although there is a certain number of studies on weight-bearing exercise effects on bone density and bone metabolism in young male athletes [16,17,18], so far much fewer studies have been conducted on highly trained male middle-aged and elderly athletes. The existing studies on male masters athletes usually do not combine bone measures with metabolic parameters [19,20]. Moreover, sparse studies that join elements of competitive sport participation, bone density/mineral content and metabolic parameters in males relate only to endurance trained masters athletes [21,22]. As yet, research of this kind, including also speedpower trained masters athletes, has not been undertaken.

Therefore, the aim of the study was to assess bone mass density and bone turnover in male masters athletes still involved in competitive sport, representing different training profile in young age and adulthood, and exposed to dissimilar competition requirements. The track and field athletics encompasses a variety of events and thus is a good example for this purpose. We compared BMD, bone mineral content (BMC), and bone turnover between endurance runners, speed-power athletes and non-sport controls.

## Methods

The study was performed in 26 white male masters track and field athletes, participants of the European Veterans Athletics Championships Stadia, and 13 non-athletic controls. The age of all subjects ranged from 40 to 64 years. They declared good health status. Subjects with inflammatory disorders, recent infections, diabetes mellitus, renal or hepatic insufficiency, anorexia nervosa, smoking and using hormonal therapy were not included into the study.

## Athletes

Athletes were divided into two categories according to the declared type of sport event during the competition: endurance athletes (long-distance runners  $\geq$  5000 m, n = 14) and speed-power athletes (seven sprinters  $\leq 400$  m, one high jumper, one long jumper, two pentathlonists, one hammer thrower, total n = 12). Four endurance athletes and four speed-power athletes were current medalists of European championships. All of them were regular participants in international athletic championships. One endurance athlete and three speed-power athletes were not participating in competitive sport in the young age. All other athletes were participating in the same sport (athletics) and in the same kind of event, i.e. current endurance runners were endurance runners before the age of 30, and speed-power athletes specialised in the same event or events of the same character in the past and at present (shifts within speed-power events were possible, e.g. between sprint and jumping). Detailed division by age in categories 40-45, 46-50, 51-55, 56-60 and 61-64 was: 3, 7, 2, 2 and 0 subjects in endurance runners; 5, 2, 1, 1, and 3 subjects in speed-power athletes; 2, 7, 1, 2 and 1 subjects in controls, respectively. Thirteen endurance runners and nine speed-power athletes participated in competitive sport before the age of 30. Athletes reported current training frequency at least four times a week.

## Controls recruitment

Control subjects were professionally active people, volunteers recruited during the European Veterans Athletic Championships Stadia. In total, 130 of volunteers were examined. Inclusion criteria were (1) age, body mass, body height and body mass index (BMI) as similar as possible to athletes' characteristics, (2) good health status, (3) low level of physical activity (<2 h/week) and (4) lack of competitive sport history in the past and at present. Selected 13 control subjects were healthy men, participating in leisure time physical activity only  $0.8 \pm 0.8$  h per week (including resistance or weight-bearing exercise) in the year preceding recruitment and they have never trained at the competitive level.

## Health, training history and physical activity

The data regarding health and training or physical activity history were obtained by means of a short structured interview, administered by one of the researchers to each participant. The interview encompassed basic information: past and present diseases as well as medication (to exclude subjects with serious disorders and illnesses), years of competitive sport and specialisation before the age of 30, starting age of masters training, masters sport history, specialisation and sports level in masters category (places in national, European or world championships), weekly training (athletes) and physical activity (controls) duration.

#### Weight and height measurement

Weight and height were measured using certified digital medical scale WPT 60/150.O (Radwag, Radom, Poland), accuracy 0.01 kg, with mechanical measuring rod for height, accuracy 0.5 cm.

#### Bone densitometry

Dual-energy X-ray absorptiometry (DXA) measurements of total-body and regional (arms, trunk, thoracic spine, pelvis and legs) aBMD, total-body, arms and legs BMC, soft tissue composition including total fat and lean body mass (total, legs and arms) were acquired on a Lunar Prodigy Advance densitometer (GE Lunar Corp., Madison, WI, USA), software enCORE 2006, using the standard whole body protocol. Reproducibility of DXA data was not assessed in this study, but is normally in the order of 1% (% CV) in our laboratory. The reproducibility of the regional analysis of the Lunar Prodigy device was reported by Wacker et al. [23]. They scanned 39 subjects (mean age 56.7 years, SD 13.7; mean BMI 25.3) in triplicate for total body. Precision values (% CV) were 0.76% for total body BMD, and 1.68% for total body BMC. Total body composition precision values were 1.74% for per cent fat, 1.64% for fat mass (g), and 1.15% for lean mass (g). Other studies, conducted on younger adults, reported precision 0.64 at the total body BMD, 0.64–0.90 for total body BMC, 0.41-0.88 for fat mass (g), 1.57-4.49 for lean mass (g), and 0.7-1.7 at the spine [24-28].

Data obtained from DXA were averaged from the right and left limbs. All scans were taken by the same technician on the same machine. The Lunar device was calibrated daily. Quality control of the DXA scanner was undertaken following the manufacturer's instructions, and analysis of the scans was done with the integrated software following the manufacturer's recommendations.

## Biochemical analysis

Blood was collected between 8 and 10 a.m. after 12 h of fasting and 24 h without strenuous exercise. Samples were centrifugated at 5000 rpm and 4°C. Serum was separated and stored at  $-70^{\circ}$ C. Serum concentrations of bone turnover markers: osteocalcin (OC) as bone formation marker and C-terminal telopeptide of type I collagen (CTX) as bone resorption marker were determined by an immunoenzymatic ELISA method using tests of Quidel Corporation (USA) and Nordic Bioscience (Denmark), respectively. Coefficients of variation were 6.6% (within-assay) and 8.5% (between-assay) for OC, and 5.5% and 8.1% for CTX, respectively.

Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), a member of a group of cytokines, was used as a marker of systemic inflammation. The concentration of TNF- $\alpha$  was measured by an immunoenzymatic high-sensitivity ELISA method and high sensitivity test of Bender MedSystems Inc. (Austria). Precision values were 8.5% for within-assay variation and 8.5% for between-assay variation. Level of total testosterone (TT) in serum was determined using radioimmunoassay by Orion Diagnostica (Finland), % CV 4.3% for intra-assay and 8.1% for inter-assay variation. Concentrations of FT, IGF-1 in serum were analysed by radioimmunological method using BioSource (Belgium) kit, within-assay precision 5.7% and between-assay precision 6.2% for FT, and 2.9% and 5.1% for IGF-1, respectively. Biochemical analyses were performed in the analytical laboratory certificated ISO:9001:2008.

The study protocol was approved by the Ethics Committee for Human Research at The Poznań University of Medical Sciences, and all participants gave their informed consent.

#### Statistical analysis

The Shapiro-Wilk test was used to check the data for normal distribution and the Levene's test for homogeneity of variance in each group of athletes and control subjects. As most variables were normally distributed and homogenous, parametric methods were applied for the whole analysis. Comparisons between three groups of subjects (endurance, speedpower and controls) for age, physical activity, somatic parameters, bone densitometry, and biochemical indices were made using one-way analysis of variance (ANOVA) and Scheffé-test as a post hoc analysis. Comparisons between both groups of athletes with respect to the training characteristics were done with T-test. A general linear model was used for each measure of aBMD and BMC as the response variable, and subject group (endurance athletes/speed-power athletes/controls) as main factors. Age, weight and height were included as covariates. Using analysis of covariance (ANCOVA), we obtained adjusted means and differences. The Pearson correlation coefficient was calculated in order to find associations between measured variables. All calculations were carried out with Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA).

#### Results

Descriptive characteristics of the examined groups of subjects are given in Table I. Athletes and controls did not differ significantly as regards age, height and BMI, although endurance runners were shorter by about 3 cm and had lower BMI value by about 2 kg/ cm<sup>2</sup> than speed-power athletes and controls. Endurance runners had significantly lower body mass (p < 0.05) than other subjects. Significant

differences were also observed in per cent fat mass (p < 0.001). Definitely higher fat content was revealed in controls (22.3%): almost twice more than in each group of athletes (about 12%). The highest level of lean body mass was shown in speed-power athletes (65151 g), who differed significantly from both endurance runners and controls by about 7000 g (p < 0.05). The mean duration of sport participation in young age was very similar in both groups of athletes (about 11 years). The mean starting age of masters training (at the age of 35), the mean duration of masters sport participation (almost 15 years), and mean total competitive training experience (26 years) also did not differ significantly between the two groups of athletes. The only training characteristic that was significantly different between athletes was weekly training volume, which was higher in endurance runners than in speed-power athletes (p < 0.05).

Table II contains crude mean values of whole body and regional aBMD and total BMC. The one-way ANOVA showed significant differences between three groups in all mean values of total and regional aBMD. The post hoc Scheffé-test revealed significantly higher values in speed-power athletes than in endurance athletes and controls for total-body aBMD (by 8.9% and 10.7%, respectively), arms (by 11.5% and 10.5%, respectively), trunk (by 11.8% and 9.5%, respectively), thoracic spine (by 13.9% and 10.6%, respectively), pelvis (by 12.1% in both cases) and legs aBMD (by 10.5% and 14.5%, respectively). Mean aBMD values in endurance athletes and control subjects were very similar and did not differ significantly. Similar significant differences were observed in crude mean values of total body and legs BMC. Total body BMC was higher in speed-power athletes than in endurance runners and controls by 14.7% and 14.1%, and legs BMC by

Table I. Mean values ± SD of age, somatic parameters and training characteristics in endurance athletes, speed-power athletes and controls. ANOVA refers to comparisons between all three groups of athletes and controls, T-test refers to comparisons between two groups of athletes.

				<i>p</i> -level
	Endurance $(n = 14)$	Speed-power $(n = 12)$	Controls $(n=13)$	ANOVA/T-test*
Age (years)	$49.2\pm5.4$	$50.6 \pm 9.2$	$49.4\pm5.7$	n.s.
Weight (kg)	$69.8\pm6.2^{\dagger,\S}$	$78.8 \pm 13.9^\ddagger$	$77.6\pm9.0^{\ddagger}$	< 0.05
Height (cm)	$176.0\pm4.5$	$179.6 \pm 9.0$	$179.0 \pm 4.2$	n.s.
Body mass index (kg/m <sup>2</sup> )	$22.5 \pm 1.3$	$24.3\pm3.2$	$24.2 \pm 1.9$	n.s.
Fat mass (%)	$11.5\pm4.3^{\dagger}$	$12.6\pm7.3^\dagger$	$22.3\pm4.6^{\ddagger,\$}$	< 0.001
Lean body mass (g)	$58912 \pm 4412^{\$}$	$65151 \pm 8534^{\dagger,\ddagger}$	$57707 \pm 5206^{\$}$	< 0.05
Training experience before the age of 30 (years)	$11.0 \pm 4.5$	$11.5\pm8.0$	-	n.s.
Starting age of masters training (years)	$34.6\pm7.4$	$35.8\pm6.9$	-	n.s.
Masters training experience (years)	$14.6\pm7.6$	$14.8\pm9.5$	-	n.s.
Total training experience (years)	$26.6 \pm 10.6$	$26.3\pm9.5$	-	n.s.
Training volume (h/week)	$7.3\pm1.5^{\$}$	$6.2\pm1.2^{\ddagger}$	_	< 0.05

n.s., not significant.

\*ANOVA for age and somatic characteristics (three groups of subjects), T-test for training characteristics (two groups of athletes). <sup>†</sup>Significantly different from controls.

<sup>‡</sup>Significantly different from endurance athletes.

<sup>§</sup>Significantly different from speed-power athletes.

	Endurance	Speed-power	Controls	One-way ANOVA			
	(n=14)	(n=12)	(n = 13)	<i>p</i> -level	Effect size	Statistical power ( $\alpha = 0.05$ )	
aBMD total-body (g/cm <sup>2</sup> )	$1.23\pm0.08^{\ddagger}$	$1.34 \pm 0.10^{\star,\dagger}$	$1.21\pm0.09^{\ddagger}$	< 0.01	0.31	0.94	
aBMD arms (g/cm <sup>2</sup> )	$1.04\pm0.12^{\ddagger}$	$1.16\pm0.14^{\star,\dagger}$	$1.05\pm0.12^{\ddagger}$	< 0.05	0.18	0.66	
aBMD trunk (g/cm <sup>2</sup> )	$0.93\pm0.06^{\ddagger}$	$1.04\pm0.09^{\star,\dagger}$	$0.95\pm0.06^{\ddagger}$	< 0.001	0.34	0.97	
aBMD thor. spine $(g/cm^2)$	$1.01\pm0.08^{\ddagger}$	$1.15 \pm 0.14^{\star,\dagger}$	$1.04\pm0.08$	< 0.01	0.27	0.89	
aBMD pelvis (g/cm <sup>2</sup> )	$1.16\pm0.09^{\ddagger}$	$1.30 \pm 0.12^{\star,\dagger}$	$1.16\pm0.10^{\ddagger}$	< 0.05	0.30	0.93	
aBMD legs (g/cm <sup>2</sup> )	$1.43\pm0.12^{\ddagger}$	$1.58 \pm 0.13^{\star,\dagger}$	$1.38\pm0.11^{\ddagger}$	< 0.001	0.35	0.97	
Total-body BMC (g)	$3070\pm309^{\ddagger}$	$3521 \pm 551^{\star,\dagger}$	$3086\pm307^{\ddagger}$	< 0.01	0.22	0.79	
Arms BMC (g)	$454 \pm 47$	$504 \pm 76$	$455 \pm 52$	n.s.	0.14	0.53	
Trunk BMC (g)	$891 \pm 135^{\ddagger}$	$1084 \pm 231^\dagger$	$948 \pm 126$	< 0.05	0.20	0.72	
Legs BMC (g)	$1286 \pm 118^{\ddagger}$	$1453 \pm 219^{\star,\dagger}$	$1231 \pm 126^{\ddagger}$	< 0.01	0.28	0.90	

Table II. Crude mean values  $\pm$  SD of whole body and regional aBMD and BMC in endurance athletes, speed-power athletes and controls.

n.s., not significant.

\*Significantly different from controls.

<sup>†</sup>Significantly different from endurance athletes.

<sup>‡</sup>Significantly different from speed-power athletes.

13.0% and 18.0%, respectively. Mean value of trunk BMC was also the highest in speed-power athletes (by 21.7% and 14.3%, respectively) but a significant difference was noted only between endurance and speed-power athletes. In spite of the tendency toward higher arms BMC in speed-power athletes compared to other groups (by 11.0% and 10.8%, respectively), significant differences were not shown.

After adjusting aBMD and BMC values for age, weight and height, the obtained picture of adjusted means and differences turned out to be more uniform (Table III). All bone parameters differed significantly between examined groups. The highest values were observed always in speed-power athletes, exceeding considerably those of endurance runners and controls who did not differ between each other. Differences expressed as percentage of speed-power athletes' values were larger for controls (from 9.6% for trunk BMD to 17.8% for legs BMC) than for endurance athletes (from 6.7% for total body BMC to 9.4% for arms aBMD).

Table IV contains mean values of biochemical indices in both athletic groups and controls. Concentrations of OC, CTX, TT, FT, TNF- $\alpha$  and IGF-1 did not significantly differ between all investigated groups. However, the variability of these parameters

was very high, and the statistical power of ANOVA very low.

The significant positive correlation between legs aBMD and total training experience (r=0.57, p < 0.05) in endurance runners was found. There were revealed no relationships between total/regional aBMD and biochemical indices in all investigated groups.

#### Discussion

In this study, total and regional aBMDs and contents were compared as well as levels of biochemical markers of bone turnover and hormonal indices were assessed to evaluate the bone metabolism in 40–64 year old masters athletes.

#### Bone parameters

The comparison showed that aBMD values of the whole skeleton, of the examined regions (spine, trunk, pelvis, arms, legs) as well as BMC values (total-body, arms, trunk, legs) were significantly higher in speed-power athletes compared with endurance athletes and controls, for both crude and adjusted values, mostly on a very high level of

Table III. Age-, weight- and height-adjusted mean values  $\pm$  SE of whole body and regional aBMD, and BMC in endurance athletes, speed-power athletes and controls.

					ANCO	VA
	Endurance $(n = 14)$	Speed-power $(n=12)$	Controls $(n=13)$	<i>p</i> -level	Effect size	Statistical power $(\alpha = 0.05)$
aBMD total-body (g/cm <sup>2</sup> )	$1.24\pm0.02^{\ddagger}$	$1.33 \pm 0.02^{\star,\dagger}$	$1.20\pm0.02^{\ddagger}$	< 0.01	0.33	0.94
aBMD arms (g/cm <sup>2</sup> )	$1.06\pm0.03^{\ddagger}$	$1.16\pm0.03^{\star,\dagger}$	$1.04\pm0.03^{\ddagger}$	< 0.05	0.17	0.58
aBMD trunk (g/cm <sup>2</sup> )	$0.95\pm0.02^{\ddagger}$	$1.03\pm0.02^{\star,\dagger}$	$0.94\pm0.02^{\ddagger}$	< 0.01	0.33	0.95
aBMD thor. spine (g/cm <sup>2</sup> )	$1.05\pm0.02^{\ddagger}$	$1.13 \pm 0.03^{\star,\dagger}$	$1.03\pm0.02$	< 0.05	0.23	0.77
aBMD pelvis (g/cm <sup>2</sup> )	$1.19\pm0.03^{\ddagger}$	$1.29\pm0.03^{\star,\dagger}$	$1.15\pm0.03^{\ddagger}$	< 0.01	0.29	0.88
aBMD legs (g/cm <sup>2</sup> )	$1.44\pm0.03^{\ddagger}$	$1.57\pm0.03^{\star,\dagger}$	$1.37\pm0.03^{\ddagger}$	< 0.001	0.37	0.97
Total-body BMC (g)	$3220\pm74^{\ddagger}$	$3435\pm77^{\star,\dagger}$	$3021\pm73^{\ddagger}$	< 0.01	0.32	0.93
Arms BMC (g)	$472 \pm 13^{\ddagger}$	$494\pm13^{\star,\dagger}$	$447 \pm 12^{\ddagger}$	< 0.05	0.17	0.60
Trunk BMC (g)	$964\pm30^{\ddagger}$	$1040 \pm 32^{\star,\dagger}$	$918\pm 30^{\ddagger}$	< 0.05	0.20	0.68
Legs BMC (g)	$1322 \pm 31^{\ddagger}$	$1422 \pm 32^{\star,\dagger}$	$1207\pm31^{\ddagger}$	< 0.001	0.42	0.99

n.s., not significant.

\*Significantly different from controls.

<sup>†</sup>Significantly different from endurance athletes.

<sup>‡</sup>Significantly different from speed-power athletes.

Table IV. Biochemical parameters (mean  $\pm$  SD) in endurance athletes, speed-power athletes and controls.

				One-way ANOVA		
	Endurance $(n = 14)$	Speed-power $(n = 12)$	Controls $(n=13)$	p-level	Effect size	Statistical power ( $\alpha = 0.05$ )
OC (ng/ml)	$10.90\pm3.56$	$11.78 \pm 2.94$	$12.00\pm4.00$	n.s.	0.02	0.10
CTX (ng/ml)	$0.66 \pm 0.29$	$0.74\pm0.25$	$0.74\pm0.33$	n.s.	0.02	0.10
TT (nmol/l)	$18.34 \pm 3.59$	$20.08 \pm 6.75$	$18.08 \pm 6.06$	n.s.	0.03	0.12
FT (pg/ml)	$8.81 \pm 2.64$	$8.80\pm3.24$	$7.46\pm5.93$	n.s.	0.02	0.12
TNF-α (pg/ml)	$0.96 \pm 1.99$	$0.34\pm0.39$	$0.23 \pm 0.15$	n.s.	0.07	0.28
IGF-1 (ng/ml)	$153.02\pm72.87$	$144.69\pm56.05$	$143.44\pm51.62$	n.s.	0.01	0.06

n.s., not significant.

statistical significance (Table II and III). The limitation of our study is that the regional analysis of aBMD cannot be supported by separate scans for loaded sites, e.g. proximal femur and lumbar spine. In an overview of cross-sectional studies, Suominen [7] has demonstrated higher BMD and BMC in middle-aged males from various sports in several fragments of the skeleton in comparison with non-athletes. The greatest differences in bone mineral mass have been observed in sites rich in trabecular bone, but the differences were lower in elderly.

Our middle-aged endurance runners did not differ significantly from the control group as regards adjusted aBMD and BMC. In younger endurance runners, a lower mineral density in lumbar spine and/or proximal femur [4,29] and in distal parts of lower limbs [30] has been demonstrated in comparison with athletes running shorter distances or even with non-exercisers. In other studies on older athletes, differences concern the most loaded sites during running (lower limbs) as a rule. In several studies, no significant differences or lower level in lumbar vertebrae, lumbar spine and forearm aBMD in middle-aged to older distancerunning males have been found compared to individuals who run less or not at all, whereas significant differences in more loaded sites (legs, femoral neck, proximal femur, trochanterion, Ward's triangle, calcaneus) have been revealed [4,19,21,22,31,32]. Similar locations (lower limbs, calcaneus, femoral neck, pelvis) were connected with the differences between young competitive male runners and controls [33,34].

Beside the location of the mechanical load, the running volume also seems to be an important factor, affecting bone density and mass. Very high volume masters runners usually do not differ from controls or have lower BMD or BMC than non-sport controls, and the negative association remains significant even when the model is corrected for body size. The volume threshold is about 90-95 km a week: above this training load no further increases are observed in BMD and BMC [5,22,30]. In our research, running volume in kilometers was not collected and the lack of differences between runners and controls cannot be interpreted in these terms. We presume that the above-mentioned volume threshold was not exceeded by our subjects, because they run on average 7.3 h a week, i.e. less than high (9.1 h/week, 69 km/week) and very high volume runners (10.6 h/week, 101 h/week) in the study of MacKelvie et al. [22]. This presumption is reinforced by the tendency toward higher values of adjusted aBMD and BMC in endurance athletes than in controls in our study. This suggests a small but positive training response as a result of a running volume below the 'risky' threshold.

The higher values of bone mass in speed-power athletes compared to distance runners may result

from the differences in training type in young age continued in masters sport, specific to a given type of physical exertion. Speed-power athletic events are characterised by short duration, extremely high intensity (maximum and supermaximum), and large, impulsive loads on bone. The largest mechanical loads come from jumping down from a height or from working muscle contractions [35]. Although we did not collect detailed information on the structure of training loads of athletes, we suppose that speedpower athletes used much more heavy and vigorous exercises (e.g. jumping, weight exercises) in their training than endurance runners did. Conzelmann [36] has revealed considerable differences in the structure of trainings loads between best German distance runners and sprinters aged 45-70. First and foremost, over 60% of sprinters developed regularly their strength and power, and almost a half of them used additional external resistance. The athletes devoted on average 0.61-1.48 training lessons per week to strength exercises, depending on training phase and specialisation. Moreover, 33-55% of sprinters practiced jumping exercises (0.31-1.06 training lessons per week). In contrast to the sprinters, only 10% of long-distance runners used any training form other than running (strength, flexibility, coordination, general fitness). In endurance runners who participated in resistance training at least twice-a-week, Hind et al. [37] have demonstrated greater lumbar spine aBMD than in athletes using only endurance training. Sprint running and depth or drop jumps (jumping down from a height) influence significantly skeleton load and, consequently, bone osteogenic reaction. Studies in men have shown that the workload is of greater importance for increase in bone mass than number of repetitions [38]. However, Karlsson [39] has reported that loading on mature bone is no more effective than normal daily use.

We found the correlation between legs aBMD and lifetime training experience in endurance runners but not in speed-power athletes. The results of similar studies are divergent. Daly and Bass [40] have detected no relationships between the lifetime total time spent participating in sport and leisure activities with any bone parameter. However, the time of participation in weight-bearing activities was an important determinant of bone size, quality and strength, but not areal or volumetric BMD at loaded sites in older men. They concluded that participation in weight-bearing exercise in early to mid-adulthood appears to be an important component of improved bone size and strength in old age. Suominen and Rahkila [31] have observed no relationship between training years and volumetric BMD as well as BMC of calcaneus in much older athletes aged 70-81 (endurance, strength and speed). Wiswell et al. [20] have found that hip and spine aBMD are maintained over a 4- to 5-year period in master runners ranging in age from 40 to 80 years old. They concluded that

bone density can be maintained by running in older active men.

## Biochemical parameters

Using biochemical markers of bone turnover, hormonal indices and TNF- $\alpha$ , we tried to understand the mechanism responsible for exercise-related effects on bone mass. Differences in bone turnover were not observed in our study. OC levels (marker of bone formation) as well as CTX levels (resorption marker) were not significantly different between groups (Table III). We also found no differences in hormonal indices levels (TT, FT) between endurance and speed-power athletes and no relationships between training volume and bone metabolic status in the both groups of athletes. The main limitation of our results is a high variability of these parameters resulting in a very low statistical power of ANOVA (effect size ranged from 0.01 to 0.07, statistical power from 0.06 to 0.028) and thus the results must be interpreted with caution.

Except for the above-mentioned obvious shortcoming, there are two other possible explanations of the lack of differences. First, training experience of studied athletes before the age of 30 (on average 11 years) indicates that they started training at their young age. Therefore, the influence of training on hormonal status and bone tissue metabolism in the earlier lifetime can not be excluded. Hetland et al. [5] have demonstrated increased (by 20-30%) levels of bone turnover markers in high volume running athletes (>100 km/week) compared to controls. Concentrations of bone turnover markers were positively related to the weekly running distance. However, endurance athletes in our study differ from Hetland's participants in age (athletes in our study are older by 16 years on average). In contrast, Brahm et al. [21] have revealed lower levels of bone formation and bone resorption markers in serum in runners than in controls by 18.0% and 22.2%, respectively. But it is to stress that the age range of subjects was very wide (19-54 years old).

Exercise in the older age plays a lesser role in increasing bone mass in comparison with childhood or maturation [41]. Frost [8] has reported that physical activity does not significantly increase bone mass in ageing adults, partly due to age-related decrease in cellular responsiveness to hormones, and fewer stem cells to create the osteoblasts needed to add bone. Bennell et al. [29] have demonstrated, like in our study, similar levels of bone metabolic markers in endurance and power athletes, despite differences in bone mass indicating the importance of childhood physical activity. This supports the hypothesis that differences in bone mass arise during the young years.

Secondly, the phase of the 1-year training cycle, when biochemical assessment in our athletes was done, could also play a role in the metabolic status. It can be supposed that athletes in our study were already adapted to the specific training loads and competition demands at the moment of the examination, which was the competition period. Thus, the bone metabolism was no longer intensified. Changes of bone biochemical markers could occur in earlier phases of the training cycle, in the preparation period, when the trainings loads (especially training volume) are increasing. The variation in markers of bone formation along with the training periods of the 1-year cycle has been observed in young adult endurance runners [42]. Further investigation is needed to find if similar variation occurs also in masters athletes.

In the present study, no significant differences were found in levels of investigated metabolic parameters like TNF-a, IGF-1 and testosterone between studied groups. The interpretation is difficult due to a high variability of these parameters. TNF- $\alpha$  is a member of a group of cytokines and a marker of systemic inflammation. Pathologic bone resorption is mediated largely by increased production of cytokines [43]. There is an increased production of TNF- $\alpha$  during ageing. TNF- $\alpha$  mediates both survival and cell death signals. There are suggestions that the measurement of TNF- $\alpha$  may give a picture of the mechanism regulating bone ageing [44]. Elosua et al. [45] have demonstrated that men aged 65 or more practicing moderate and high intensity physical activity had significantly lower concentrations of TNF- $\alpha$  than sedentary men. Regular training may also alter the secretion of IGF-1 [10]. Poehlman and Copeland [46] have shown that lower levels of IGF-1 with ageing in men are related to diminished physical activity. Arii et al. [47] have revealed significantly higher IGF-1 levels in masters athletes aged  $68 \pm 6$  than in sedentary controls. Also, a lack of connection between IGF-1 concentration and physical performance has been reported [48,49]. Cooper et al. [48] have found comparable plasma IGF-1 level in elderly long-term endurance-trained master runners and sedentary elderly men.

MacKelvie et al. [22] have revealed no differences in TT and FT between high volume runners and controls; however the within group variances were very high, like in our study. Although testosterone levels were negatively associated with weekly training volume, the reported values fell within the normal healthy range and no relation to BMD has been found. Suominen and Rahkila [31] have also demonstrated no correlation between BMD and serum TT. However, a significant increase in testosterone levels has been observed in both young and older men following a 10-week strength-power training program [9], indicating that also older men can make physiological adaptations in the endocrine system with resistance training, but the plasticity of the system was limited compared to younger subjects. Tissandier et al. [10] have observed only

the tendency (p = 0.08) to higher levels of FT in old trained male subjects.

In summary, this study showed, in line with previous reports [4,22,29], that power-speed athletes have greater aBMD than endurance athletes. This suggests that weight-bearing exercise with large loading forces in young age and the training continuation in later life appears to be an important contributor to the aBMD in the middle age and in the elderly. In addition to this, there was not any difference in bone resorption, bone formation markers, serum levels of IGF-1, TNF- $\alpha$  and testosterone. This would suggest that differences in bone between power-speed athletes, distance runners and controls are not caused by present alterations in bone turn-over or somatotropic effects. However, a very high variability of biochemical parameters, which resulted in low power of statistical analysis, considerably limits this conclusion. Much larger samples are needed to detect the relationship between bone and metabolic parameters. Our earlier study on young endurance runners has revealed the changeability of bone metabolic indices during the 1-year training cycle [42]. Therefore, an investigation of bone turnover markers during longer training periods in middle-aged and elderly male athletes is recommended for future research. Moreover, the BMD measurement of more sensitive sites like femoral neck or lumbar spine should be included in the analysis to assess the bone parameters in athletes.

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