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Original Article

Karate Training Improves Skeletal Status Assessed by Quantitative Ultrasound in Girls and Premenopausal Women

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Abstract

Purpose: The aim of the study was to assess the influence of regular karate training on the skeletal status evaluated by quantitative ultrasound (QUS) in females. Methods: A group of 132 karate training girls and women at mean age 19.57 (standard deviation [SD] 7.64) yr (range 7.3–45.3 yr) and 322 age-, sex- and body size-matched controls were enrolled into the study. Mean training duration in the karate group was 7.52 (SD 5.05) yr and mean training frequency was 2.97 (SD 1.21) per wk. The QUS measurements were performed at hand proximal phalanges, using a DBM Sonic 1200 (IGEA, Carpi, Italy) sonographic device, which measures amplitude-dependent speed of sound (Ad-SoS [m/s]). Results: The results of Ad-SoS obtained in karatekas were generally higher than in controls with significant difference for prepubertal girls (1966.2 [SD 46.2] vs 1942.7 [SD 38.4]; p < 0.05) and for adult women (2124.4 [SD 48.0] vs 2105.3 [SD 54.0]; p < 0.05). Conclusions: Regular karate training is a factor that is positively associated with results of the QUS measurements at hand phalanges in exercising females and its impact is most strongly pronounced in prepuberty and adulthood.

Key Words: Bone; Female; Karate training; Quantitative ultrasound.

Introduction

Status of the bone tissue is one of the crucial factors determining the general health and quality of life throughout an individual's life. The effects of the reduced strength of bones, manifested mainly as pathological fractures, are particularly evident and severe in the elderly and affect women to greater extent (1,2). This raises the need to efficiently and effectively identify and treat subjects suffering from osteoporosis. However, primary prevention, understood as measures conducive to achieving

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*Address correspondence to: Piotr Adamczyk, PhD, Department of Pediatrics, Medical University of Silesia in Katowice, 3-Maja 13/15 street, 41-800 Zabrze, Poland. E-mail: adamczyk@konto.pl a higher peak bone mass and slowing its physiological decrease in pre and postmenopausal women, may be much more important.

Regular physical exercise is one of the factors with known positive effects on bone health. Much attention was paid to this issue in studies conducted in postmenopausal women, aimed at verification, if physical activity is sufficient to diminish the postmenopausal bone mass loss (3-5). Consistent findings derived from those publications are the basis of universal recommendation of regular physical activity in postmenopausal women. Physical activity is also one of established physiological determinants of peak bone mass value achieved at a young adult age (6). Moreover, bone loading together with nutrition, are potentially modifiable factors in contrast with unmodifiable (sex, race, and heredity) or hardly modifiable (hormonal status) factors. Literature regarding the effects of physical activity on the skeleton in females during the developmental period and in premenopausal women is also quite numerous (7-12).

It can be assumed that the modifications of bone properties are strongly dependent on the specific type of physical training. Specifically, bone loading exercises are expected to modify the mechanical properties of bone tissue and support effective bone mass accretion leading to higher peak bone mass. Such effects were previously proven for female tennis and squash players (13, 14). Most of such studies are based on dual-energy X-ray absorptiometry (DXA). A recently published study presented the influence of karate training on the quantitative ultrasound (QUS) bone measurements in a wide age range of males (15). The research supported the hypothesis that karate training effected in bone loading, leading to a higher and sustained bone mass. The QUS method may be considered as a reasonable option in the evaluation of bone changes related to physical training in young subjects due to its totally noninvasive character and potential for assessment of both quantitative and qualitative changes in bone structure (16,17). In experimental studies it has been proven that OUS parameters can be predictors of mechanical properties of the bone independent of bone density (18). The detailed characteristics of QUS performed at hand phalanges describing the factors influencing the result, as well as the precision of the method, has been provided in study published by Njeh et al (19). QUS measurements at hand phalanges were previously applied in different clinical conditions, such as: anticonvulsant treatment in children (20), hormonal changes related to aging in men (21), diabetes in postmenopausal women (22), development of girls with Turner syndrome (23) or fractures during childhood and adolescence (24), and many others.

The aim of the current study was to evaluate, with the QUS method, the influence of regular karate training on bone tissue in prepubertal girls, female teenagers, and premenopausal adult women.

Methods

A group of 132 prepubertal girls, adolescent girls, and premenopausal adult women (age range 7.3–45.3 yr), who had participated in regular karate training for at least 6 mo, were enrolled into the study. They were recruited from various karate clubs of the Polish Karate Association performing in several cities of the Silesia region. Data concerning the studied subjects were collected in a special questionnaire (age, body size, the age at the start of karate training, its duration, and frequency). Only subjects without bone metabolism affecting diseases, longterm medication usage, and prior fracture(s) were included in the study. For some analyses the study group was divided into 3 subgroups established due to physiological periods of ontogeny (prepuberty, puberty, and adulthood).

A control group including 322 age-, sex-, and body size-matched healthy counterparts was selected from previously published study (25).

The study design was approved by the Ethics Committee of the Medical University of Silesia (Katowice, Poland). All of the adult participants and the children's parents/caregivers provided an informed written consent prior to enrolling in the study.

QUS Bone Measurements

Skeletal status of study participants was assessed with DBM Sonic 1200 (IGEA, Carpi, Italy) device. The DBM Sonic device consists of 2 probes: one is the emitter and the other the receiver of ultrasound waves, and both are mounted on an electronic caliper. Acoustic coupling was achieved by using a standard ultrasound gel. Amplitude-dependent sound speed (Ad-SoS [m/s]) was measured at distal metaphysis of the proximal phalanges of fingers second—fifth of both hands, taking into account the first signal with an amplitude of at least 2 mV at the receiving probe. Thus, the measured speed of sound is specified as "amplitude dependent." The mean value of the 4 fingers was then calculated (separately for both hands) and used in further analyses.

T-score and Z-score values were also calculated using the reference values from a previously published population-based study (25). T-score presents the number of standard deviations (SDs) from the peak Ad-SoS value achieved in the referenced population, while Zscore presents the number of SDs from the mean Ad-SoS value calculated for age- and sex-matched cohort derived from the referenced population.

The in vivo precision was previously established based on repeated measurements in 70 women, measured 5 times each with the repositioning. The coefficient of variation (%) was 0.70% (25). All the measurements performed in the subjects from both the study and the control groups were done by the same experienced operator (WP) and with the same device calibrated before each measurement session, in accordance with the manufacturer's recommendations.

Statistics

The statistical analysis was performed using the Statistica software (StatSoft, Tulsa, OK). Descriptive statistics were presented as mean values and SDs. The normality of data distribution was verified by the Shapiro-Wilk test. Comparisons between results of the dominant and nondominant side were performed by t test for dependent variables. The differences between the studied subjects and controls, as well as between subgroups derived from the study group, were established by the Student's t test or the Mann-Whitney Utest (in the case of data that have a normal distribution and data that do not have a normal distribution, respectively). Some comparisons were also verified with ANCOVA analysis, with age as a covariate. Correlation analysis was done by Spearman's correlation. A multivariate analysis regarding the identification of independent factors influencing the QUS parameters was performed by applying multiple stepwise regression. Significance of the results obtained was assumed at p < 0.05.

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Results

Clinical characteristics of the karate training subjects and selected controls are presented in Table 1.

QUS Results in Karatekas Vs Controls

The mean Ad-SoS value in the whole group of female karatekas measured at phalanges in the dominant hand was 2075.8 (SD 84.1) m/s, and in the nondominant hand 2075.5 (SD 84.3) m/s. Since the measurements for the dominant and nondominant sides did not differ significantly, and the subjects from the control group were previously examined solely at their dominant side, only the values for the dominant hand were taken into account when calculating either the T-scores or Z-scores, as well as all further analyses.

The comparison between the whole study and control groups revealed that the Ad-SoS and T-score values were nonsignificantly higher in karatekas (2075.8 [SD 84.1] m/s in karatekas vs 2064.7 [SD 80.3] m/s in controls [p > 0.05] for Ad-SoS and -0.88 [SD 1.61] in karatekas vs -1.11 [SD 1.64] in controls [p > 0.05] for T-score, respectively), whereas the Z-score was significantly higher in karatekas than in controls (0.20 [SD 1.13] vs -0.02 [SD 1.00]; p < 0.05]. A reanalysis of the comparison between subjects and controls performed by the ANCOVA analysis, with age as a covariate, confirmed the significance only in the case of Z-score (p < 0.05).

Taking into consideration the assumption that the influence of physical training may differ at consecutive stages of an individual's development (prepubertal period, adolescence, and adulthood), additional comparisons between karatekas and controls divided into age categories were performed. Those results are presented in Fig. 1. It is noticeable that karate training increases the values of QUS measurements in the youngest girls and in adult women, whereas during the period of adolescence there is no difference between girls in training and controls. In the adult subgroup, the older women are compared, the more pronounced the difference between their results is observed.

Age-Related Ad-SoS Changes in the Studied Karatekas and in the Controls

Comparisons between karatekas and controls based on age categories suggested a discrepancy in age-related Ad-SoS changes in both groups. The expected phenomenon is shown in Fig. 2 which presents the relationship between age and Ad-SoS for compared groups. The Ad-SoS vs age curves were plotted as polynomial trendlines. It can be noticed that the increasing trend of the Ad-SoS value, typical for the developmental period, achieves plateau in the control group at around 25 yr of age, and clearly begins to decrease afterward. However, in the training group, the age-related Ad-SoS plateau is only reached at around 35 years of age, and the downward trend starting after that age has a lower deterioration rate than in the control group.

 Table 1

 Clinical Characteristics of Studied Subjects and Controls (Mean [SD])

| Parameter | Karatekas (n = 132) | Controls $(n = 322)$ |
|---|-----------------------------|-----------------------------|
| Age (yr) Weight (kg) | 19.57 (7.64) 55.2 (13.4) | 19.59 (8.49) 55.0 (14.1) |
| Height (cm) | 161.0 (12.3) | 160.3 (13.5) |
| BMI (kg/m ²) Duration of training (yr) | 20.9 (3.2) 7.52 (5.05) | 21.0 (3.8) |
| Frequency of training per wk | 2.97 (1.21) | - |

No significant differences for mean age and body size between the karatekas and the controls were noted.

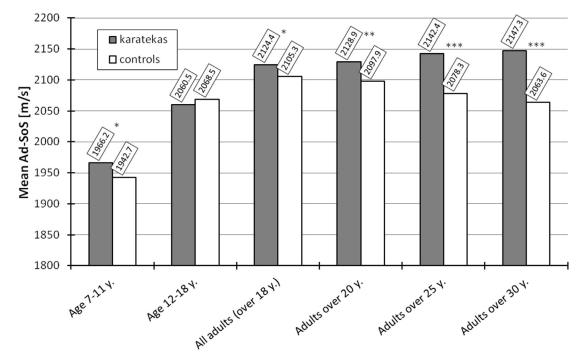
The Influence of Karate Training Duration and Frequency on QUS Parameters

The correlation analysis revealed that there is a significant, positive correlation between Ad-SoS and the time since one begins karate training (r = 0.51; p < 0.0001), and the frequency of trainings (r = 0.47; p < 0.0001). However, as both training duration and frequency are strongly correlated with the age of the studied subjects, the correlation analysis was also performed with Z-score instead of Ad-SoS, which provided the age-adjustment. When Z-score was applied, the correlation with training duration and frequency became much weaker (r < 0.1 for both) and insignificant. The comparisons between longtime training karatekas (more than 10 years of training) and the rest of the cohort, as well as between frequently training karatekas (more than 4 trainings per wk) and the rest of the cohort, did not reveal any significant differences (data not shown).

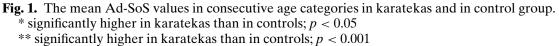
The Influence of Beginning Karate Training Early, on the QUS Parameters

It was additionally checked if beginning karate training early lead to a prolonged impact on the QUS measurements. The cohort was divided according to the age at the beginning of the trainings into an "early beginner" subgroup (n = 80, onset of trainings before the age of 12 yr, mean ageat onset of trainings 8.52 [SD 2.03] yr) and a "late beginner" subgroup (n = 52, onset of trainings after the age of 12 yr,mean age at onset of trainings 17.50 [SD 5.77] yr). The average time of trainings did not differ significantly between those subgroups (7.87 [SD 5.51] yr for early beginners vs 6.96 [SD 4.23] yr for late beginners). Obviously, the early beginners during the study were significantly younger than the late beginners (16.39 [SD 6.22] yr vs 24.46 [SD 7.06] yr; p < 0.0001), so only the Z-score was used for comparison between the subgroups. The Z-score in early beginners was 0.07 (SD 1.10) and did not differ significantly from the Zscore for the late beginners (0.40 [SD 1.16]), revealing no

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Age categories for comparisons between karatekas and controls



*** significantly higher in karatekas than in controls; p < 0.0001.

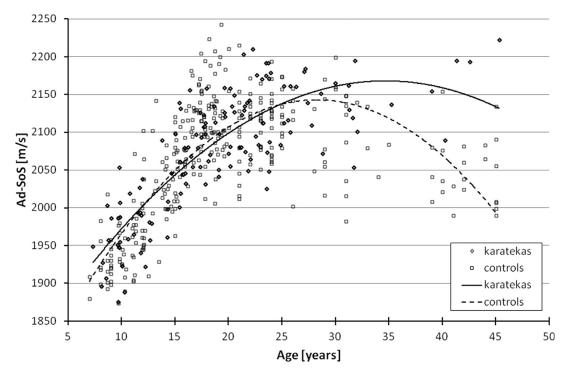


Fig. 2. Relationships between Ad-SoS and age in the whole study cohort of karatekas and in the controls.

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sustained positive effect on the obtained QUS results for early beginning of karate training.

The Influence of Menarcheal Status on QUS Parameters

As the data on the age of menarche were also gathered during the study (in karatekas only, not in controls), some additional analyses referring to the pubertal status instead of a priori established age categories were performed. There were 25 girls at the premenarcheal age in the study group. Their results were compared with 37 postmenarcheal teenagers. As the compared subgroups were definitely different in mean age, only the Z-scores were employed for analysis. In a direct comparison the mean Z-score in premenarcheal girls was significantly higher than in menstruating teenagers (0.45 [SD 1.34] vs -0.19 [SD 1.00]; p < 0.05). However, the ANCOVA analysis, performed with age as a covariate, revealed that the difference in the Z-score between pre and postpubertal girls is insignificant. This suggests that the pubertal (or to be exact: menarcheal) status does not have any direct influence in modifying the effectiveness of karate training on QUS bone measurements in young girls and teenagers.

It was also possible to calculate if karate trainings were initiated before or after menarche. The comparison between athletes starting their trainings before or after menarche was in turn limited to adult subjects—in order to establish the possible long lasting effect of premenarcheal beginning of karate training on obtained results. Among 70 adult karatekas, there were 29 women who started their training before menarche, and 41 women beginning their training after menarche. The clinical characteristics of those 2 subgroups of adult karatekas and the results of direct comparisons between their QUS results are given in Table 2. Those findings could suggest that there is no (theoretically expected) benefit for the bone quality resulting from early (premenarcheal) beginning of karate training in women continuing their physical activity during adulthood. Perhaps postmenarcheal beginning may even provide a better long-term outcome. However, the recomparison with the ANCOVA tool, with age as a covariate, allowed to reject the latter hypothesis suggesting that the observed differences (both for Ad-SoS and Z-score) are related to age only, and not to the menarcheal status.

Multiple Stepwise Regression Analyses

As the QUS parameters in karate training females may be influenced by both physiological factors common for the healthy population and some specific factors related to repeated regular physical activity, multiple stepwise regression analyses were performed as well. Regarding the expected differences for the Ad-SoS trend in the developmental and adulthood periods, the regression analysis was conducted separately for girls/adolescents and for adults. The Ad-SoS and Z-score values were regressed on age, body mass, height, training duration, and training frequency. The results of multiple stepwise regression analyses are presented as regression equations in Table 3.

It can be summarized that during the developmental period (prepubertal girls and adolescents together), the QUS results are determined positively by training frequency and negatively by body mass and training duration. In adults, a positive influence of age (not only for Ad-SoS, but also for Z-score) and height, as well as a

| (Mean [SD]) | | | | |
|------------------------------------|--|---|---------------------------------|--|
| | Trainings started before menarche n = 29 | Trainings started after menarche n = 41 | <i>p</i> value (<i>t</i> test) | |
| Age (yr) | 23.14 (4.74) | 26.36 (6.63) | < 0.05 | |
| Age at beginning of trainings (yr) | 9.98 (2.06) | 18.30 (6.25) | < 0.0001 | |
| Age at menarche (yr) | 12.96 (1.02) | 12.73 (1.00) | NS | |
| Weight (kg) | 61.6 (8.0) | 61.1 (7.8) | NS | |
| Height (cm) | 166.0 (6.7) | 166.7 (5.2) | NS | |
| $BMI (kg/m^2)$ | 22.3 (2.3) | 21.9 (2.2) | NS | |
| Duration of trainings (yr) | 13.15 (4.69) | 8.06 (3.85) | < 0.0001 | |
| Frequency of training per wk | 4.1 (1.3) | 3.0 (1.1) | < 0.001 | |
| Ad-SoS (m/s) | 2110.3 (42.5) | 2134.4 (49.7) | < 0.05* | |
| T-score | -0.18(0.87) | 0.31 (1.01) | < 0.05* | |
| Z-score | -0.02(0.86) | 0.56 (1.16) | < 0.05* | |

Table 2

Clinical Characteristics and QUS Results of Adult Karatekas Starting Their Trainings Before or After Menarche (Mean [SD])

Abbr: Ad-SoS, amplitude-dependent speed of sound; QUS, quantitative ultrasound, SD, standard deviation.

*When verified in ANCOVA analysis, the difference became insignificant.

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| | Regression equation | р | R ² | SEE |
|--------------------------------------|--|----------|----------------|------|
| Prepubertal girls and adolescents | Ad-SoS = $1759 \text{ (m/s)} + 21.7 \times \text{age (yr)}$ +20.1 × frequency (per wk) | <0.0001 | 0.70 | 40.2 |
| (n = 62) | $-1.37 \times \text{weight (kg)} -2.65 \times \text{duration (yr)}$ Z-score = $0.78 - 0.03 \times \text{weight (kg)}$ + $0.47 \times \text{frequency (per wk)}$ $-0.09 \times \text{duration (yr)}$ | <0.01 | 0.19 | 1.09 |
| Adult women (n = 70) | Ad-SoS = 1846 (m/s) + 2.67 × age (yr) -2.54 × weight (kg) + 2.2 × height (cm) | < 0.001 | 0.24 | 42.9 |
| | $Z-score = -6.45 + 0.09 \times age (yr)$ $-0.05 \times weight (kg) + 0.05 \times height (cm)$ | < 0.0001 | 0.40 | 0.87 |

 Table 3

 Multiple Stepwise Regression Analyses for the Karateka Females

Abbr: Ad-SoS, amplitude-dependent speed of sound.

negative influence of body mass are noticeable; whereas parameters related to the training intensity (frequency and duration) remain insignificant. Especially interesting is the finding regarding a positive influence of age on the QUS results in adults, since in the control group (like in the general population) the relation between Ad-SoS and age during adulthood is significantly negative (detailed regression equations for the controls are not presented).

Discussion

To the best of the authors' knowledge, the current study is the first attempt to show the effects of karate training on the skeleton in a group of girls and women. As a diagnostic method, QUS has been applied, which allows to assess both quantitative and qualitative characteristics of a bone (16-19). The QUS results are dependent not only on bone tissue calcification (like in the case of absorptiometry), but also on such features as bone elasticity and cortical bone thickness (16-19).

The unavailability of studies carried out by other authors in a similar study group of female karatekas makes direct comparisons with our results impossible. It seems to be most fitting to refer the findings in female karate group to a previously published study, which was designed similarly and carried out in male karatekas (15). In the current study we found significantly higher values of Ad-SoS in adult karatekas in comparison to the controls, and no significant difference in the adolescent subgroup, which is coherent with the observation made in male karatekas. Such dependence of Ad-SoS variability on the age category may suggest that changes in properties of the bone tissue during puberty are mainly determined by physiological factors common for study and control groups (i.e., physical development and hormonal changes), and that they are not very sensitive to additional modifying factors, such as physical training. The same was indicated by Dib et al (26), who found no influence of physical activity on the QUS measurements in a group of 256 children and adolescents, aged 11-18 years (including girls and boys). However, some papers published by other authors indicate that training may lead to improved bone mass acquisition in adolescent girls, but the effect is strongly dependent on the type of sport. Positive effects were identified in girls practicing football and artistic gymnastics, however a weaker effect was observed for rhythmic gymnasts, and no effect was detected for swimmers (8,9).

Considerable difference between adult female karatekas and controls suggests a significant positive impact of karate training on the skeletal health in women after the end of adolescence. Several aspects of that influence may be specified: achieving a higher peak bone mass, extending the period until achieving peak bone mass (from the age of about 25 yr to approximately 35 yr), and a smaller rate of decrease in bone mass after that age. A very convincing observation is the positive relationship between Ad-SoS and age, revealed in multiple regression analysis. It shows the reverse trend of Ad-SoS vs age in comparison to women not involved in regular physical training-those from our control group, as well as from the populationbased studies (25). Of course, the described relationship is, to some extent, determined by the fact that our current study group included only premenopausal women (up to the age of 45 yr) Therefore, that finding cannot be simply extrapolated to the whole period of adulthood. Higher QUS results in the karate group, already evident in the third and fourth decade of life, are also interesting due to the observation that other forms of activity manage to demonstrate a clear positive influence on the skeletal health usually only for postmenopausal women (27-30). It can be assumed that early karate training provides a good basis for effective preservation of high bone mass after menopause, supported by other forms of physical activity, e.g., Tai Chi practice, which is becoming more and more popular among the elderly. Obviously, the difference in the QUS results between female karatekas and controls (see Fig. 1), cannot be interpreted as a different level of

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fracture risk. The T-score and Z-score for Ad-SoS are calculated according to the same mathematical formula as the T-score and Z-score for BMD measurements, but it does not mean that they have the same clinical significance as the T-score and Z-score in DXA results. The dynamics of age-related changes in the QUS results is different in comparison to DXA. For QUS measurements there is no evident plateau between the age of "peak" results (25–30 yr) and menopause (25). In our study we are just presenting the different trends in age-related changes of Ad-SoS in female karatekas and controls with "normal" physical activity, without any suggestion about the discrepancy in fracture risk between those groups.

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An interesting phenomenon found in the current study, and not recorded in the case of karate-training young boys, are the significantly higher (in comparison to age-matched controls) Ad-SoS values in the subgroup of prepubertal karate-girls. It is known from the population-based study (25) that skeletal maturation in girls starts its rapid acceleration after the age of 11. Guided by this criterion, the authors decided to extract subgroups of the youngest girls (aged 7–11 yr) and adolescents for a separate analysis. Surprisingly, it was shown that only the youngest girls exercising karate had the QUS values significantly higher than their nontraining counterparts. This also suggests that the prepubertal period of ontogeny in females is characterized by bone tissue susceptibility to the influence of some factors positively modifying skeletal development.

Taking into consideration the sensitivity of bone tissue to the effects of training in prepuberty discussed above, it was interesting to also check whether early initiation of trainings (before the age of 12 yr) modifies the results of the QUS in later years. There was no evidence, however, for such an effect—the results for girls and women who had taken karate training before puberty did not differ from those who had started to train at a later age.

The findings were similar when the age of menarche (instead of *a* priori established by metrical age category thresholds) was applied for gathering subjects into subgroups for comparisons. In an analysis limited to young girls and adolescents (with adult athletes excluded) which focused on the "current" influence of trainings on bone tissue, the direct comparison of premenarcheal and postmenarcheal girls suggested that stronger effects were achieved in prepubertal girls. However, the ANCOVA analysis identified the menarcheal status as an insignificant factor. In a study conducted in a group of female tennis players, the connection between puberty and the effectiveness of training on bone density was not very evident (14). In contrast to our study, some significant differences between athletes and controls were detectable for girls above Tanner stage III only (which corresponds with postmenarcheal stage of puberty). However, it should be noticed that tennis represents a type of sport with unilateral activity and that also a different diagnostic tool (DXA) was applied.

What is more interesting is the verification of the hypothesis that a very early beginning (premenarcheal) in training may lead to intensive bone mass accretion with long-lasting positive effects, also detectable much later. Such phenomenon was suggested in a study performed in middle-aged female tennis and squash players, who had started training before, at or after menarche (13). Premenarcheal beginning of regular physical activity was recognized as beneficial. In our cohort, this hypothesis was not confirmed. In a direct comparison, the results of adult karatekas who started their training before menarche, seemed to be even worse than the results of adults with postmenarcheal beginning physical activity. ANCOVA excluded the significance of the menarcheal status in that comparative analysis. Again, the characteristics of specific sports (karate vs squash or tennis, which may induce mainly unilateral development) and different diagnostic tools applied in the discussed studies (QUS vs DXA) may be responsible for the inconsistent findings. It may be summarized on the basis of our own studies that the pre or postmenarcheal status at the beginning of karate training is not very important and that the crucial issue is to undertake and continue with the training at any age category.

It can be stated that we did not find any significant influence of training frequency and duration, nor an early beginning of training, on the QUS results. On the other hand, the difference in QUS results between karatekas and controls is statistically significant. It could be recapitulated that regular karate training leads to improvement in QUS measurements at hand phalanges, but there is no "additional benefit" resulting from high frequency or longterm training, nor from an early beginning of training.

The presented study has some limitations. Only 1 skeletal site was assessed. Karate is a sport with stronger impact on musculature and skeleton of upper limbs, which could contribute to more expressive results than it would be in case of other skeletal sites. Only 1 diagnostic tool was applied; bone densitometry was not available. No additional information about sport activity in controls or additional exercises in the karate group was available. Also, the possible differences in dietary regimen were not taken into consideration. Although the total number of participant is quite satisfactory, some of the results of subanalyses provided in subgroups may require confirmation in bigger cohorts.

In conclusion, it was shown that karate is a sport with a positive influence on the QUS measurements at hand phalanges in girls and women. The effect is strongly pronounced in prepubertal girls and in adults. During adolescence the physiological pattern of bone development in examined skeletal site seems to be modified by karate training to a lesser extent. In the adulthood, the difference between trainees and controls is growing up with increasing age of subjects suggesting the bigger benefit in the fourth decade of life.

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Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jocd.2018.07.008.

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