

Original Article

Forearm bone density, cross-sectional size and muscle cross-sectional area in adolescents with diabetes mellitus type 1 assessed by peripheral quantitative computed tomography

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Abstract

Objectives: The mechanical components of bone strength (size, shape and density) in adolescents with T1DM are not extensively studied. **Methods:** The studied group comprises 39 adolescents, aged 11,9-18,0 yrs. The bone and muscle properties were investigated at the forearm (66% and 4% site). All measurements were performed using pQCT method. **Results:** The mean Z-score calculated for the ratio of the total cortical bone cross-sectional area to muscle cross-sectional area at 66% was lower than zero in girls (-0,93+/- 1,06; p=0,0042). Significant differences between Tanner stages were noted in boys for mean Z-scores for bone masses, cross-sectional dimensions and strength. **Conclusions:** T1DM girls revealed a decreased ratio of cortical bone area/muscle area, reflecting disturbed adaptation of the cortical shaft to the muscle force. When the Z-scores of cortical shell dimensions were investigated, cases in Tanner stage 5 diverged from "less mature" individuals, which may suggest that bone shaft development in these individuals was impaired, affecting both size and strength.

Keywords: T1DM, Adolescent, Bone Mineral Density, Forearm, pQCT

Introduction

Diabetes mellitus type 1 (T1DM) is known as a disease that may affect bone metabolism in children^{1,2}. Studies performed in children of different ages documented bone abnormalities, as measured by pQCT in the forearm³⁻⁶. However, there

is no agreement on how T1DM impacts bone and what specific features of the bone are impaired. Most likely, the limited knowledge on the bone status and the specific bone abnormalities during T1DM course is related to the fact that, most of the studies published so far focused only on selected features of the bone. On the other hand, one study done in adolescents, suggested that the bone status in T1DM cases improves and normalizes with age⁷. Nonetheless, it is still unknown whether the normalization concerns all aspects of the bone properties such as shape, architecture and its adaptation to the forces generated by muscles^{8,9}.

Peripheral quantitative computed tomography (pQCT) allows to measure bone mineral density in 3-D cross-sectional images of long bones at certain levels, with a discrimination between the trabecular and the cortical compartment¹⁰⁻¹². It is also possible to measure the bone area and the circumference of the cortical layer as well as the muscle cross-sectional area at the same time. Moreover, pQCT is able to examine the whole bone shape in the meaning of the ratio of bone areas between certain levels¹³. Giving the

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Table 1. Characteristics of studied group by sex.

Gaussian distributed variables					
	Female		Male		p value ⁽¹⁾
	Mean	SD	Mean	SD	
Height [cm]	158,1	6,59	176,5	8,38	<0,000001
Weight [kg]	53,4	9,95	66,3	8,33	0,00017
BMI [kg/m ²]	21,2	3,30	21,2	2,00	0,98
HbA _{1c} mean [%]	7,61	0,85	7,70	1,58	0,83
Z-score height	-0,62	0,97	0,36	1,09	0,0094
Z-score BMI	0,44	0,79	0,16	0,72	0,26
Non-normally distributed variables					
	Female		Male		p value ⁽²⁾
	Median	Quartiles (Q1 – Q3)	Median	Quartiles (Q1 – Q3)	
Age [yrs]	14,6	12,9 – 17,2	16,4	15,0 – 17,6	0,20
Age at diagnosis [yrs]	10,7	8,8 – 13,0	12,1	8,6 – 14,5	0,38
Time since diagnosis [yrs]	4,9	1,8 – 6,2	3,0	1,3 – 5,7	0,70
Z-score weight	-0,25	-0,55 – 0,96	0,28	-0,14 – 0,62	0,38

Female n=15; Male n=21. BMI – body mass index. HbA_{1c} – Glycated haemoglobin. ⁽¹⁾ Student's t test. ⁽²⁾ Mann-Whitney test.

Table 2. Number of individuals by Tanner stage and sex.

Sex	Tanner stage 3	Tanner stage 4	Tanner stage 5	Sum
Female	6	6	3	15
Male	5	8	8	21
Sum	11	14	11	overall n=36

Table 3. Daily dietary intake of selected micronutrients and energy-providing macronutrients in individuals with type 1 diabetes by sex, with adherence to the recommendations^{14,15}.

	Female (n=15)		Male (n=21)		p value* (girls vs boys)
	Mean (SD)	No. in norm (and in %)	Mean (SD)	No. in norm (and in %)	
Calcium [mg]	588 (249)	1 (6,7%)	758 (329)	2 (9,5%)	0,10
Phosphorus [mg]	978 (212)	12 (80,0%)	1348 (267)	3 (14,3%)	0,000087
Magnesium [mg]	222 (47,9)	5 (33,3%)	297 (62,8)	6 (28,6%)	0,00050
Percent energy from proteins [%]	17,6 (2,95)	10 (66,7%)	17,6 (1,98)	18 (85,7%)	0,99
Percent energy from carbohydrates [%]	49,1 (4,08)	11 (73,3%)	49,9 (4,88)	14 (66,7%)	0,60
Percent energy from sucrose [%]	5,56 (2,28)	15 (100%)	5,25 (2,69)	20 (95,0%)	0,58 ⁽¹⁾
Percent energy from fat [%]	33,3 (3,63)	9 (60,0%)	32,5 (4,73)	13 (61,9%)	0,59
Percent energy from saturated fatty acids [%]	12,0 (2,74)	5 (33,3%)	11,5 (2,26)	6 (28,6%)	0,59
Fibre per 1000 kcal [g/1000 kcal]	10,2 (2,78)	2 (13,3%)	8,72 (2,29)	1 (4,7%)	0,047 ⁽²⁾

* - p value for comparison of mean (or median) intake in girls vs. boys. ⁽¹⁾ and ⁽²⁾ - Mann-Whitney test. ⁽¹⁾ medians are 6,18 and 4,32 for girls and boys, respectively; Q1-Q3 are 3,50-7,58 and 3,79-6,56, respectively. ⁽²⁾ medians are 9,21 and 8,76 for girls and boys, respectively; Q1-Q3 are 8,29-13,4 and 7,39-9,02, respectively.

Table 4. Laboratory profile – basic bone profile by sex.

	Female (n=15) Mean (SD)	Male (n=21) Mean (SD)	p value
25(OH)D [ng/ml]	15,6 (5,88)	16,4 (5,99)	0,68
PTH [ng/ml]	33,1 (20,7)	28,9 (13,6)	0,62 ⁽¹⁾
Ca serum [mmol/L]	2,40 (0,072)	2,47 (0,107)	0,075
P serum [mmol/L]	1,41 (0,23)	1,31 (0,18)	0,17

⁽¹⁾ - Mann-Whitney test; medians are 27,8 and 27,1 for girls and boys, respectively; Q1-Q3 are 17,9-47,6 and 16,8-34,7, respectively.

fact that the effective dose for a patient is very low, less than a daily dose received from natural sources of radiation^{10,11,13}, pQCT is a suitable method for an extensive assessment of various skeletal properties bone in children and adolescents.

The purpose of this study was to assess the bone mineral density, cross-sectional size, longwise bone shape and bone/muscle ratio in adolescents with diabetes mellitus type 1.

Studied group

The participants were recruited from patients treated in the Department of Endocrinology and Diabetology. Inclusion criteria were as follows: age 12-18 yrs, diagnosis of diabetes mellitus type 1 according to International Society for Pediatric and Adolescent Diabetes criteria, duration of diabetes and received medical services received in the clinic for at least six months. All individuals were treated by continuous subcutaneous insulin infusion. The exclusion criteria were as follows: history of any acute (severe hypoglycaemia, diabetic ketoacidosis) or chronic (retinopathy, neuropathy, nephropathy, bone pain or fracture) complications of diabetes, the presence of any associated metabolic bone or musculoskeletal diseases, and any chronic illness other than diabetes as well as any medications other than insulin. Three individuals with Tanner stage 2 were excluded from the study due to their incompatibility to the entire group. Finally, the studied group comprised of 36 adolescents (15 girls), aged from 12,3 to 17,9 yrs. The characteristics of the studied group are presented in Tables 1, 2, 3 and 4.

The study was conducted according to the Declaration of Helsinki and with a permission of the local Ethics Committee (Warsaw, Poland). Informed written consents were obtained from parents of the participants.

Methods

Biochemistry

Blood samples were collected between 7:00 a.m. and 9:00 a.m. after an overnight fasting. HbA1c levels were analyzed using a direct turbidimetric inhibition immunoassay that determines HbA1c as a percentage of the total haemoglobin. The mean HbA1c level was defined as a mean value from the last year (for individuals with a diabetes duration of one year or longer), or a mean value from the 3 last measurements

(for individuals with a diabetes duration shorter than one year). The serum levels of calcium and inorganic phosphorous were measured spectrophotometrically using commercially available test kits (Roche Diagnostics, Germany). Serum total 25(OH)D and intact PTH (iPTH) were measured by a direct electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Germany), according to the manufacturer's instructions.

Tanner stage

The Tanner stage was assessed by physicians as a part of a routine diagnostic procedure.

Dietary assessment

The usual dietary intake was measured using a standardized 3-day food records. Individuals were requested to record everything they ate and drank for three consecutive days which included two week days and one weekend day. Total daily energy and nutrient intakes were calculated using dietary software DIETA v. 6.0¹⁶ based on the national food composition database. Results were referred to the national dietary reference intake recommendations¹⁴ and to the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines¹⁵.

Peripheral Quantitative Computed Tomography

All measurements were done on non-dominant forearm¹². Stratec XCT 2000L (Stratec Medizintechnik, Pforzheim, Germany) apparatus with software v. 6.20 was used. Forearm length was measured with the ruler from the ulnar styloid process to the olecranon. The scout view was used to determine start position as follows: if the growth plate was visible the reference line was placed through the most distal portion of the growth plate; if the growth plate had fused the reference line was placed through the middle of horizontal part of the articular surface of the radius. The scan lines were automatically placed at a distances of 4% and 66% of the forearm length, proximal to the reference line¹³. Scan speed, slice thickness and voxel size were 30 mm/s, 2,3 mm and 0,5x0,5 mm, respectively. At the 4% site trabecular volumetric bone mineral density (mg/cm³), total volumetric bone mineral density (mg/cm³) and total bone cross-sectional area (mm²) were measured. CALCBD algorithm was used,

Table 5. pQCT Z-scores in comparison with hypothetical mean zero by sex.

	Female (n=15) Mean (SD) p value (diff. from 0)	Male (n=21) Mean (SD) p value (diff. from 0)	p value (diff. between female and male)
Bone mineral densities			
Z-score radius 4% trabecular bone density	0,25 (0,76) (0,23)	-0,58 (1,04) (0,018)	0,013
Z-score radius 4% total bone density	-0,01 (1,39) (0,98)	-0,53 (1,25) (0,068)	0,25
Z-score radius 66% cortical bone density	0,16 (1,07) (0,57)	0,52 (1,07) (0,037)	0,33
Bone masses			
Z-score radius 4% bone mass	-0,12 (1,04) (0,67)	0,18 (1,22) (0,51)	0,45
Z-score radius 66% bone mass	-0,67 (1,31) (0,076) ⁽¹⁾	0,20 (1,12) (0,43)	0,019 ⁽³⁾
Cross-sectional dimensions			
Z-score radius 4% total bone cross-sectional area	-0,09 (1,47) (0,82)	0,51 (1,32) (0,089)	0,21
Z-score radius 66% inner cortical bone circumference	-0,12 (1,16) (0,68)	-0,04 (1,34) (0,89)	0,84
Z-score radius 66% outer cortical bone circumference	-0,38 (1,30) (0,28)	0,07 (1,20) (0,78)	0,29
Z-score radius 66% cortical shell thickness	-0,47 (1,35) (0,20)	0,11 (1,06) (0,63)	0,16
Z-score radius 66% cortical bone cross-sectional area	-0,69 (1,39) (0,077)	0,15 (1,14) (0,56)	0,057
Z-score radius 66% total bone cross-sectional area	-0,43 (1,24) (0,20)	0,08 (1,23) (0,76)	0,23
Longitudinal shape indexes			
Z-score radius 66% cortical bone cross-sectional area/ radius 4% total bone cross-sectional area	-0,56 (1,15) (0,082)	-0,40 (1,08) (0,11)	0,67
Z-score radius 4% bone mass/ radius 66% bone mass	0,50 (0,97) (0,068)	-0,13 (1,03) (0,57)	0,074
Strength strain index			
Z-score radius 66% polar SSI	-0,50 (1,29) (0,068) ⁽²⁾	0,38 (1,24) (0,17)	0,032 ⁽⁴⁾
Muscle and bone			
Z-score forearm 66% muscle cross-sectional area	0,26 (0,78) (0,22)	0,34 (1,10) (0,17)	0,80
Z-score forearm 66% total cortical bone cross-sectional area/muscle cross-sectional area	-0,93 (1,06) (0,0042)	-0,30 (0,92) (0,15)	0,066

⁽¹⁾ and ⁽²⁾ - one sample Wilcoxon test. ⁽¹⁾ - median and Q1-Q3 are: -0,91 and -1,29 - -0,70. ⁽²⁾ - median and Q1-Q3 are: -0,52 and -1,60 - -0,18. ⁽³⁾ and ⁽⁴⁾ - Mann-Whitney test. ⁽³⁾ - medians are -0,91 and 0,17 for girls and boys, respectively; Q1-Q3 are -1,29 - -0,70 and -0,50 - 0,94, respectively. ⁽⁴⁾ - medians are -0,52 and 0,15 for girls and boys, respectively; Q1-Q3 are -1,60 - -0,18 and -0,08 - 1,59, respectively.

with contour mode 1, peel mode 1 and threshold of 280 mg/cm³. Area was set as 45% (central) in the case of trabecular volumetric bone mineral density determination. At the 66% site CORTBD algorithm with separation mode 1 and threshold of 711 mg/cm³ was used for determining cortical volumetric bone mineral density (mg/cm³), cortical cross-sectional area (mm²) and total bone cross-sectional area (mm²). For polar

strength strain index (SSI) (mm³) calculation threshold of 280 mg/cm³ was used. CALCBD algorithm was used with threshold 40 mg/cm³, contour mode 1, peel mode 2 and filter FO3FO5 for determination of muscle+bone cross-sectional area and with threshold 280 mg/cm³, contour mode1 and peel mode 2 for bone cross-sectional area. Muscle cross-sectional area (mm²) was calculated by subtraction of bone

Table 6. Mean Z-scores of pQCT outcomes by Tanner stage in girls.

	Tanner stage 3 Mean (SD) (n=6)	Tanner stage 4 Mean (SD) (n=6)	Tanner stage 5 Mean (SD) (n=3)	ANOVA overall p value
Bone mineral densities				
Z-score radius 4% trabecular bone density	0,31 (0,93)	0,05 (0,81)	0,50 (0,16)	0,43 ⁽¹⁾
Z-score radius 4% total bone density	0,18 (1,80)	-0,16 (1,39)	-0,07 (0,66)	0,92
Z-score radius 66% cortical bone density	-0,21 (0,76)	0,25 (1,40)	0,75 (0,86)	0,47
Bone masses				
Z-score radius 4% bone mass	-0,18 (1,28)	-0,08 (1,15)	-0,07 (0,29)	0,99
Z-score radius 66% bone mass	-0,70 (1,26)	-0,32 (1,64)	-1,29 (0,63)	0,61
Cross-sectional dimensions				
Z-score radius 4% total bone cross-sectional area	-0,12 (1,93)	0,03 (1,51)	-0,27 (0,34)	0,96
Z-score radius 66% inner cortical bone circumference	0,34 (1,02)	-0,14 (1,27)	-1,03 (0,95)	0,26
Z-score radius 66% outer cortical bone circumference	-0,06 (1,28)	-0,22 (1,49)	-1,33 (0,80)	0,39
Z-score radius 66% cortical shell thickness	-0,78 (1,19)	-0,26 (1,67)	-0,26 (1,32)	0,79
Z-score radius 66% cortical bone cross-sectional area	-0,66 (1,28)	-0,40 (1,82)	-1,31 (0,60)	0,69
Z-score radius 66% total bone cross-sectional area	-0,09 (1,15)	-0,27 (1,42)	-1,40 (0,77)	0,33
Longitudinal shape indexes				
Z-score radius 66% cortical bone cross-sectional area/radius 4% total bone cross-sectional area	-0,37 (1,39)	-0,43 (1,22)	-1,18 (0,12)	0,61
Z-score radius 4% bone mass/radius 66% bone mass	0,58 (0,29)	0,01 (1,35)	1,30 (0,43)	0,17
Strength strain index				
Z-score radius 66% SSI polar	-0,23 (1,31)	-0,34 (1,46)	-1,37 (0,74)	0,45
Muscle and bone				
Z-score forearm 66% muscle cross-sectional area	0,27 (1,00)	0,25 (0,76)	0,28 (0,58)	1,00
Z-score forearm 66% total cortical bone cross-sectional area/muscle cross-sectional area	-0,91 (1,20)	-0,61 (0,94)	-1,61 (0,98)	0,44

⁽¹⁾ - Kruskal-Wallis ANOVA; median (Q1 – Q3) is: 0,60 (0,21 – 0,94), -0,21 (-0,58 – 0,40) and 0,49 (0,35 – 0,87) for Tanner stage 3, 4 and 5, respectively.

cross-sectional area from muscle+bone cross-sectional area. Bone mass (g) was calculated as multiplication of total bone cross-sectional area by total bone density at particular bone slice. Outer cortical bone circumference, inner cortical bone circumference and cortical shell thickness were calculated basing on circular ring model with CALCBD algorithm with contour mode 1, threshold 710 mg/cm³, peel mode 2 and inner threshold 710 mg/cm³¹⁷. Finally, following ratios were calculated: radius 66% cortical cross-sectional area to radius 4% total cross-sectional area and radius 4% bone

mass to radius 66% bone mass as a measures of longwise bone shape¹³ and forearm 66% total cortical cross-sectional area to muscle cross-sectional area as a measure of bone/muscle relationship^{8,9}. Quality of each slice was inspected by the operator according to visual scale¹⁸. All slices were considered as technically valid.

Effective dose involved in the procedure is as follows: scout view – 0,08 microSv; CT scan 4% site – 0,22 microSv; CT scan 66% site – 0,22%; total dose – 0,52 microSv¹³.

All measurements were done by the same operator on

Table 7. Mean Z-scores of pQCT outcomes by Tanner stage in boys.

	Tanner stage 3 Mean (SD) (n=5)	Tanner stage 4 Mean (SD) (n=8)	Tanner stage 5 Mean (SD) (n=8)	ANOVA overall p value
Bone mineral densities				
Z-score radius 4% trabecular bone density	-0,18 (0,17)	-0,32 (1,01)	-1,10 (1,25)	0,20
Z-score radius 4% total bone density	-0,85 (0,75)	-0,08 (1,07)	-0,77 (1,61)	0,46
Z-score radius 66% cortical bone density	0,46 (0,69)	0,31 (0,93)	0,77 (1,42)	0,67 ⁽¹⁾
Bone masses				
Z-score radius 4% bone mass	1,03 (1,71)	0,55 (0,65)	-0,72 (0,73)	0,021 ⁽²⁾
Z-score radius 66% bone mass	0,94 (1,25)	0,52 (0,71)	-0,60 (0,97)	0,022
Cross-sectional dimensions				
Z-score radius 4% total bone cross-sectional area	1,45 (1,83)	0,71 (0,63)	-0,26 (1,13)	0,056
Z-score radius 66% inner cortical bone circumference	0,64 (0,50)	0,52 (0,78)	-1,03 (1,61)	0,051 ⁽³⁾
Z-score radius 66% outer cortical bone circumference	0,84 (0,89)	0,59 (0,73)	-0,92 (1,14)	0,0050
Z-score radius 66% cortical shell thickness	0,33 (0,85)	0,16 (0,75)	-0,07 (1,46)	0,80
Z-score radius 66% cortical bone cross-sectional area	1,00 (1,43)	0,36 (0,74)	-0,60 (0,90)	0,031
Z-score radius 66% total bone cross-sectional area	0,80 (0,84)	0,65 (0,73)	-0,93 (1,21)	0,027 ⁽⁴⁾
Longitudinal shape indexes				
Z-score radius 66% cortical bone cross-sectional area/radius 4% total bone cross-sectional area	-0,51 (1,24)	-0,32 (0,76)	-0,41 (1,36)	0,96
Z-score radius 4% bone mass/radius 66% bone mass	-0,01 (0,96)	0,15 (0,99)	-0,49 (1,12)	0,46
Strength strain index				
Z-score radius 66% SSI polar	1,23 (1,15)	0,84 (0,98)	-0,61 (0,86)	0,0059
Muscle and bone				
Z-score forearm 66% muscle cross-sectional area	0,97 (1,38)	0,83 (0,79)	-0,52 (0,59)	0,0093
Z-score forearm 66% total cortical bone cross-sectional area/muscle cross-sectional area	-0,13 (0,67)	-0,59 (1,01)	-0,12 (1,00)	0,55
⁽¹⁾ , ⁽²⁾ , ⁽³⁾ and ⁽⁴⁾ - Kruskal-Wallis ANOVA. Medians (Q1 – Q3) are as follows: ⁽¹⁾ 0,62 (0,23 – 0,81), 0,46 (-0,03 – 1,06) and -1,14 (-1,98 – 0,14); ⁽²⁾ 1,77 (-0,03 – 2,39), 0,56 (-0,07 – 0,92) and -0,87 (-1,09 – -0,43); ⁽³⁾ 0,66 (0,66 – 0,70), 0,45 (-0,43 – 1,04) and 0,84 (-0,13 – 1,73); ⁽⁴⁾ 1,01 (0,02 – 1,39), 0,69 (-0,02 – 1,21) and -1,07 (-1,91 – 0,22); for Tanner stage 3, 4 and 5, respectively. SSI - strength strain index.				

the same unit. Routine quality assurance procedures were carried out, basing on phantom supplied by manufacturer. The phantom comprises two “parts”: standard and cone. Standard phantom was measured at least each day when patients were measured. Cone phantom was measured monthly. Measurement errors were (CV%, standard phantom): 0,20% for total density, 0,28% for trabecular density and 0,24% for cortical density in the whole study period.

Body height (cm) and weight (kg) were measured in the standing position using stadiometer with medical scale (Tryb,

Bydgoszcz, Poland). Body mass index (kg/m²) was calculated as body weight divided by squared height. Age of each participant was calculated from birth and examination dates.

Statistics

Departures from the Gaussian distribution were assessed with using the Shapiro-Wilk test. Normally distributed variables were presented as a mean and standard deviation, while non-normally distributed variables as a median and quartiles. One sample t-test was used for testing the

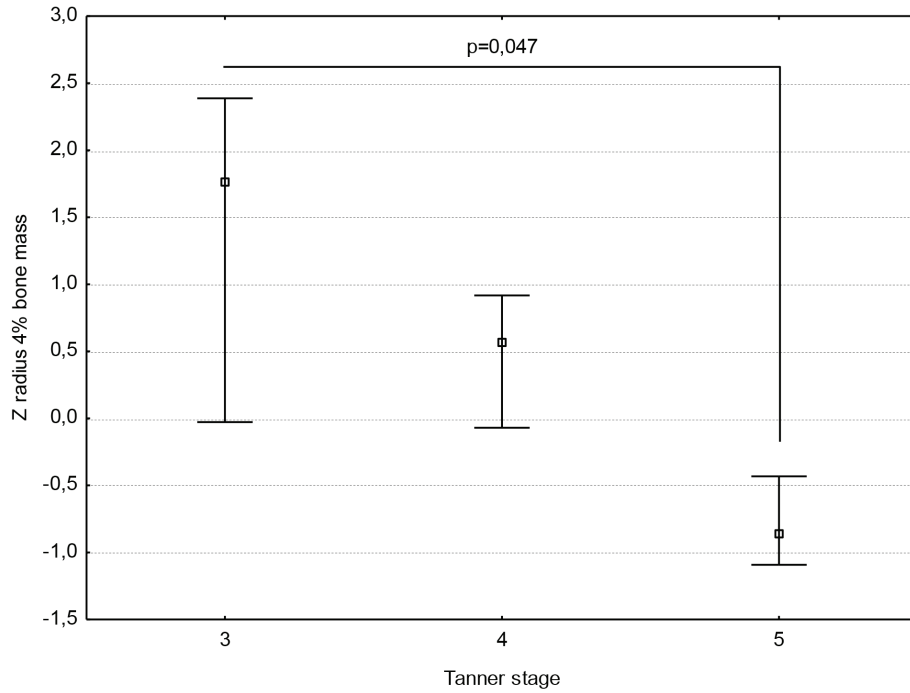


Figure 1. Median Z-scores (and Q1-Q3) for radius 4% bone mass in boys.

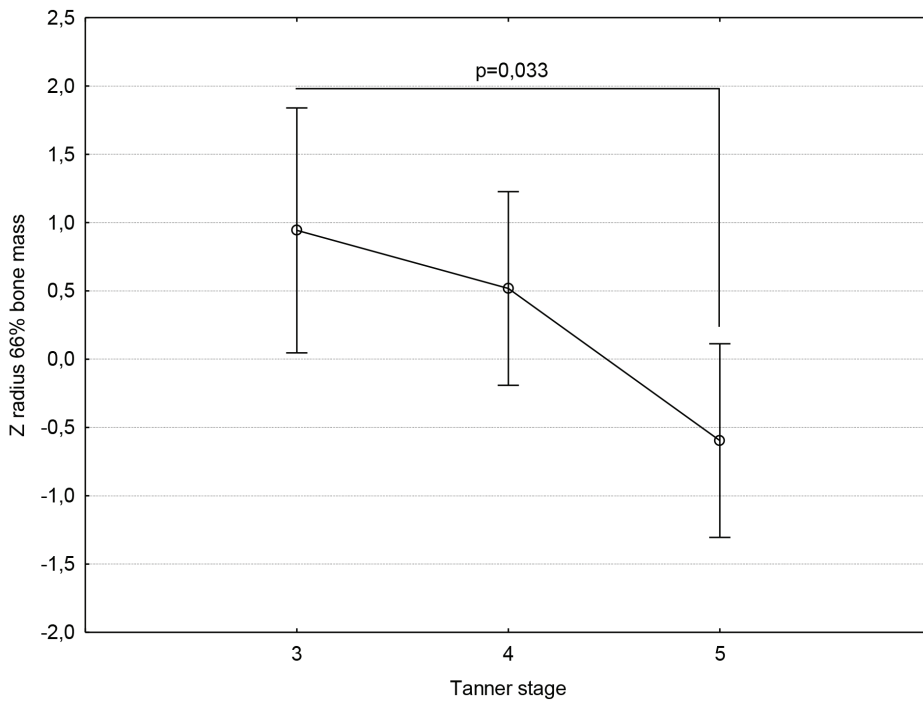


Figure 2. Mean Z-scores (and 95% CI) for Z-score radius 66% bone mass in boys.

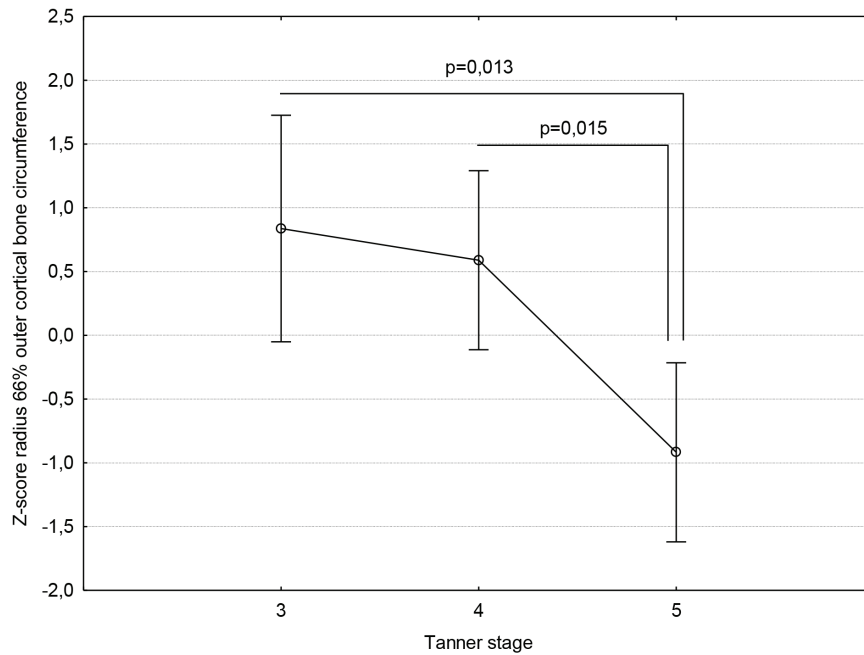


Figure 3. Mean Z-scores (and 95% CI) for radius 66% outer cortical bone circumference in boys.

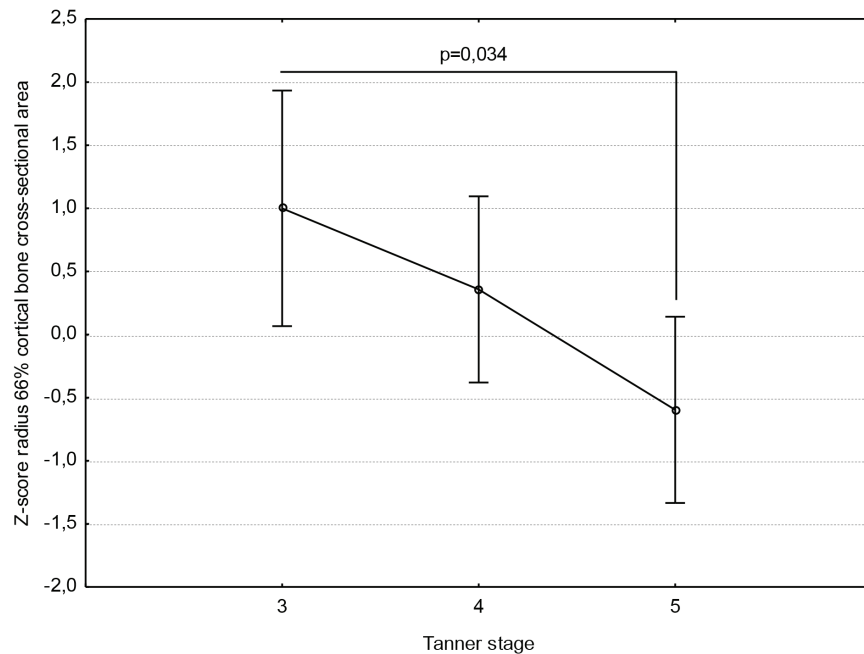


Figure 4. Mean Z-scores (and 95% CI) for Z-score radius 66% cortical bone cross-sectional area in boys.

difference between the mean Z-score and the hypothetical mean zero 0, and ANOVA with Bonferroni post-test was used for the comparison Z-scores in groups by the Tanner stage. In the case of a departure from the Gaussian distribution one

sample Wilcoxon test and the Kruskal-Wallis non-parametric ANOVA were used, respectively. Statistica v. 10 (StatSoft Inc., Tulsa, USA) was used for statistical calculations. P value less than 0,05 was considered as significant.

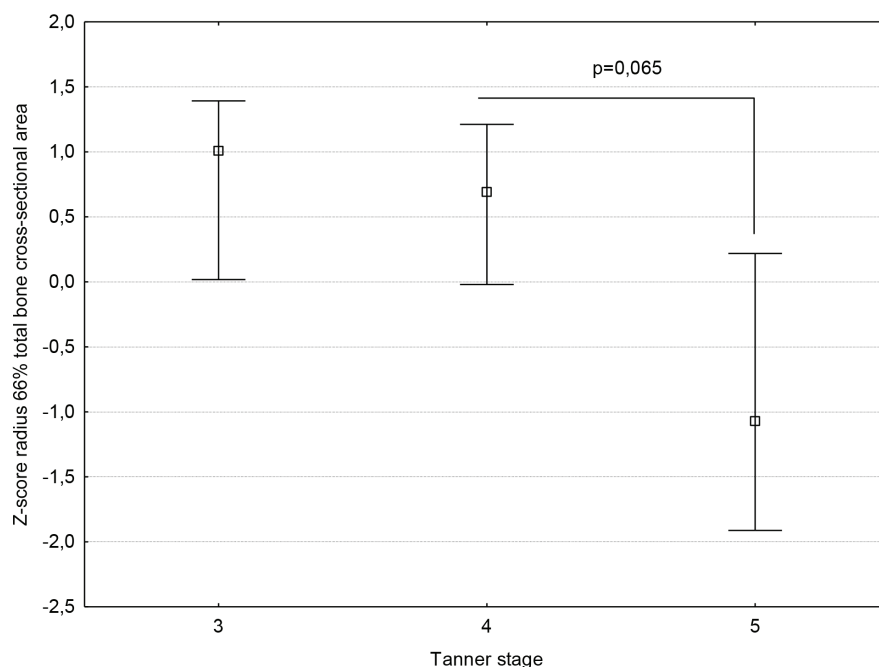


Figure 5. Median Z-scores (and Q1-Q3) for Z-score radius 66% total bone cross-sectional area in boys*. (* - overall ANOVA p is 0,027, however post-test do not reach significance level, the lowest p value is presented).

LMS Growth v. 2.77 (Medical Research Council, UK) was used for Z-scores derivations, basing on local reference data for height, weight and BMI¹⁹ as well as for pQCT outcomes²⁰.

Results

Peripheral quantitative computed tomography outcomes were measured, and Z-scores were calculated according to age and sex for each participant. The means Z-scores for all outcomes were compared with the hypothetical mean value of zero, separately for both sexes, and the difference between girls and boys were tested. The results were presented in Table 5. In the case of departure from Gaussian distribution nonparametric tests were used and median and interquartile range were also presented. In girls mean Z-scores for bone mineral densities, bone masses, cross-sectional dimensions, longitudinal shape indexes and strength strain index were not significantly different from zero as well as for muscle cross sectional area. The mean Z-score of the forearm 66% total cortical bone cross-sectional area to muscle cross-sectional area ratio mean Z-score was significantly lower than zero ($-0,93 \pm 1,06$; $p=0,0042$). In boys the mean Z-score was slightly but significantly reduced for radius 4% trabecular bone density ($-0,58 \pm 1,04$; $p=0,0018$), while for radius 66% cortical bone density mean Z-score was significantly higher than zero ($0,52 \pm 1,07$; $p=0,037$). Difference between girls and boys were noted for radius 4% trabecular bone density, radius 66% bone mass and for radius 66% polar

SSI. Mean Z-score for radius 4% trabecular bone density was significantly higher in girls than in boys, $0,25 \pm 0,76$ vs. $-0,58 \pm 1,04$, respectively. At the 66% proximal radius the bone mass and polar SSI Z-scores in girls were significantly lower than in boys, $-0,67 \pm 1,31$ vs. $0,20 \pm 1,12$ and $-0,50 \pm 1,29$ vs. $0,38 \pm 1,24$, respectively.

The studied individuals were divided into 3 groups according to the Tanner stage. Differences in the mean Z-scores between the groups were analyzed using a one-way analysis of variance (ANOVA). The overall ANOVA results are shown in Tables 6 and 7 for girls and boys, respectively. In the case of the overall p value less than 0,05, individual differences between the groups were assessed using the Bonferroni post-test. The results were presented in Figures 1-7. There were no statistically significant differences among mean Z-scores values when the Tanner stage groups were investigated in girls (Table 6). In boys statistically significant differences were observed for Z-score radius 4% bone mass, Z-score radius 66% bone mass, Z-score radius 66% outer cortical bone circumference, Z-score radius 66% cortical bone cross-sectional area, Z-score radius 66% total bone cross-sectional area, Z-score radius 66% SSI polar and for Z-score forearm 66% muscle cross-sectional area (Table 7). For radius 4% bone mass the median (and Q1-Q3) Z-scores for Tanner stages 3, 4 and 5 were 1,77 ($-0,03 - 2,39$), 0,56 ($-0,07 - 0,92$) and $-0,87$ ($-1,09 - -0,43$) (overall $p=0,021$) with statistically significant difference between Tanner stage 3 and 5 ($p=0,047$) (Figure 1). In the case of radius 66% bone mass mean (and SD) Z-scores were as follows: 0,94 (1,25),

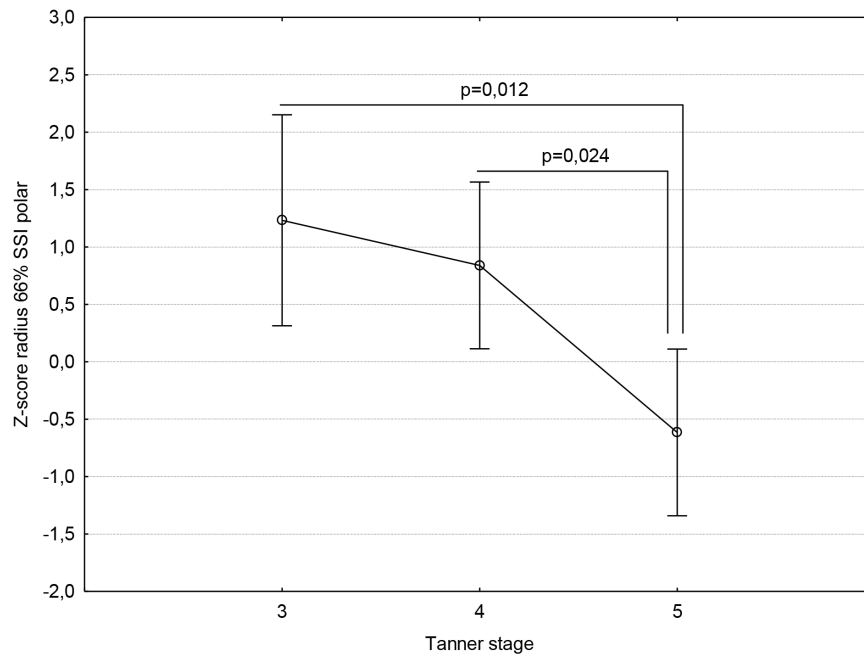


Figure 6. Mean Z-scores (and 95% CI) for Z-score radius 66% polar strength strain index in boys.

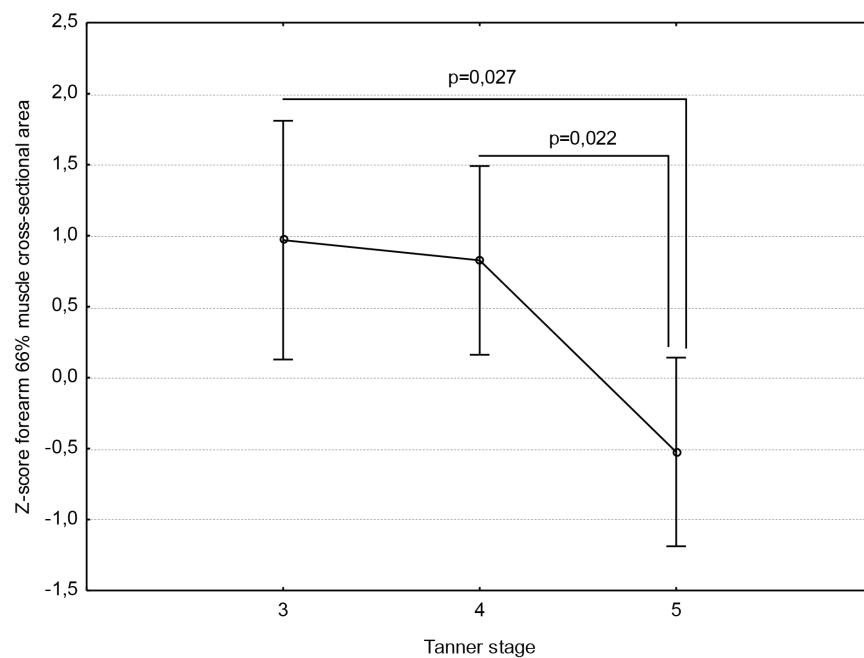


Figure 7. Mean Z-scores (and 95% CI) for Z-score forearm 66% muscle cross-sectional area in boys.

0,52 (0,71) and -0,60 (0,97) for Tanner stage 3, 4 and 5, respectively (overall $p=0,022$), with statistically significant difference between Tanner stage 3 and 5 ($p=0,033$) (Figure 2). For radius 66% outer cortical bone circumference mean

Z-scores were 0,84 (0,89), 0,59 (0,73) and -0,92 (1,14) for Tanner stage 3, 4 and 5, respectively (overall $p=0,0050$), with statistically significant difference between Tanner stage 3 and 5 ($p=0,013$) as well as 4 and 5 ($p=0,015$) (Figure 3).

In the case of radius 66% cortical bone cross-sectional area mean (and SD) Z-scores were 1,00 (1,43), 0,36 (0,74) and -0,60 (0,90), for Tanner 3, 4 and 5, respectively (overall $p=0,031$) with statistically significant difference between Tanner stage 3 and 5 ($p=0,034$) (Figure 4). For radius 66% total bone cross-sectional area median (and Q1-Q3) Z-scores were 1,01 (0,02 – 1,39), 0,69 (-0,02 – 1,21) and -1,07 (-1,91 – 0,22) for Tanner stage 3, 4 and 5, respectively (overall $p=0,027$), however post-tests p values did not reach significance level, the lowest p value was observed for comparison Tanner 4 and 5 groups ($p=0,065$) (Figure 5). In the case of radius 66% SSI polar mean (and SD) Z-scores were as follows: 1,23 (1,15), 0,84 (0,98) and -0,61 (0,86) for Tanner stage 3, 4 and 5, respectively ($p=0,0059$), with statistically significant difference between Tanner stage 3 and 5 ($p=0,012$) as well as 4 and 5 ($p=0,024$) (Figure 6). For forearm 66% muscle cross-sectional area mean Z-scores (and SD) were 0,97 (1,38), 0,83 (0,79) and -0,52 (0,59) for Tanner stage 3, 4 and 5, respectively ($p=0,0093$), with statistically significant difference between Tanner stage 3 and 5 ($p=0,027$) as well as 4 and 5 ($p=0,022$) (Figure 7).

Discussion

There are only a few papers showing results of the forearm measurements by pQCT in the T1DM children and adolescents³⁻⁷. All authors studied bone mineral density, mostly at 4% of the length of forearm. Lettgen B. et al.³ noted lowered trabecular bone mineral density in T1DM children while the total volumetric bone mineral density at the same site remained unchanged. Bechtold S. et al.⁵ found the same total volumetric bone mineral density in T1DM individuals and in controls and they noted even higher trabecular bone mineral density in T1DM individuals than in controls. Roggen I. et al.⁶ also studied trabecular bone mineral density at 4% of the forearm. They found the same trabecular BMD in both groups. In the present study, we also observe lack of decline of trabecular bone mineral density and total volumetric bone mineral density in T1DM girls at 4% distal radius, however, in T1DM boys trabecular bone mineral density was decreased. Observed decline was not severe (Z-score= -0,58+/-1,04) but statistically significant. Until now, bone mineral density measurement of radial diaphysis was described in two papers^{4,5}. Saha MT. et al.⁴ studied 1/3 distal radius (33% of the length of forearm). They found no difference between diabetic and control children in the cortical bone mineral density values. Bechtold S. et al.⁵ studied at 1/3 proximal radius (66% of the length of forearm). They found no difference between diabetic and control children in the cortical volumetric bone mineral density. In our study, we observed no decline for cortical volumetric bone mineral density in girls, while the same parameter in boys was slightly increased; mean Z-score value of 0,52+/-1,07.

Bone masses were studied only by Saha MT. et al.⁴. They found no differences between T1DM children and controls at 4% of the forearm length, while at 33% of the forearm

length they observed lowered bone mass (measured as bone mineral content) in T1DM children. In the present study, we also observed lack of decline of bone mass in T1DM children at 4% distal radius, as well as at 66% proximal radius. However, at the 66% measurement site T1DM girls revealed significantly lower Z-scores than T1DM boys. It may be originated from slightly elevated Z-scores in boys, rather than lowered Z-score in girls (-0,67+/-1,31, $p>0,05$ for comparison with zero).

Cross-sectional dimensions of bones at distal radius were studied in two papers^{4,6}. Saha MT. et al.⁴ measured total cross-sectional area at 4% length of the forearm. They found no differences between T1DM children and controls. On the contrary, Roggen I. et al.⁶ found lowered total cross-sectional area at the same measurement site, however, the decline was limited to girls only. In the present study, we also observed the lack of decline of total cross-sectional area in T1DM children at 4% distal radius, regardless of sex.

Cross-sectional dimensions of radial diaphysis were studied in two papers^{4,5}. Saha MT. et al.⁴ studied cortical bone cross-sectional area at 1/3 distal radius (33% of the length of the forearm). They found lower values of cortical bone area in diabetic children. The same phenomenon was noted by Bechtold S. et al.⁵, who studied 1/3 proximal radius (66% of the length of the forearm), for both: cortical bone cross-sectional area and total bone cross-sectional area. In our study, we observed no statistically significant decline in T1DM boys nor girls for cortical bone cross-sectional area and total bone cross-sectional area. However, in girls mean Z-score for cortical bone cross sectional area was as low as -0,69+/-1,39 but did not reach significance of difference from the value of zero. We also studied inner cortical bone circumference, outer cortical bone circumference and cortical shell thickness, which were not studied until now. No decline of Z-scores in T1DM boys and girls for bone circumferences as well as cortical shell thickness was observed. We studied two ratios which were not studied yet: 4% bone mass to radius 66% bone mass ratio and radius 66% cortical bone cross-sectional area to radius 4% total cross-sectional bone area ratio as a measures of longitudinal bone shape. The Z-scores for these indexes were not significantly declined in T1DM girls nor in boys. However, the Z-scores for radius 66% cortical bone cross-sectional area to radius 4% total cross-sectional bone area ratio were consistently slightly decreased in both sexes (-0,56+/-1,15 for T1DM girls and -0,40+/-1,08 for T1DM boys) but statistically significance level was reached only when put both sexes together (mean Z-score -0,46+/-1,10; $p=0,016$; not shown in table).

Bone strength was studied by Saha MT. et al.⁴. They applied polar section modulus calculation for 4% distal radius and 33% proximal radius. They found lowered values in T1DM children at 33% proximal radius but not at 4% distal radius. In the present study we calculated SSI at 66% proximal radius. No decline of Z-scores for SSI in T1DM girls nor in boys was shown. However, statistically significant difference between girls and boys were observed. T1DM girls tended to have lower Z-scores than boys (-0,50+/-1,29 vs 0,38+/-1,24; $p=0,032$).

In addition, we studied muscle cross-sectional area and the ratio of forearm 66% total cortical cross-sectional area to muscle cross-sectional area, as a measure of bone/muscle relationship. Muscle cross-sectional area was normal in both sexes while forearm 66% total cortical cross-sectional area to muscle cross-sectional area ratio was lowered in girls. The decline was quite remarkable, the mean Z-score was as low as $-0,93 \pm 1,06$. It may suggest that bone in T1DM girls do not adapt properly to loads generated by muscle.

To assess the impact of sexual maturity on bone development, we also analyzed bone outcomes by Tanner stages. There is no similar comparison in the literature. Roggen I et al.⁶ and Saha MT et al.⁴ applied the Tanner stage as a co-factor for matching control groups and Bechtold S et al.⁵ applied the Tanner stage as a single factor for the matching. We observed lowered values of mean Z-scores in Tanner stage 5 in boys but not in girls, for bone masses, cross-sectional dimensions and bone strength, in comparison to 3 and/or 4 Tanner stage groups. The effect could be partially explained by lowered cross-sectional muscle area in these individuals, however mean Z-score for muscle cross-sectional area was only slightly declined ($-0,52 \pm 0,59$) while Z-scores for bone masses and cross-sectional area reach lower level, from $-0,61$ to $-1,03$. Lowered bone masses, dimensions and strength are inconsistent with Bechtold's S. et al. observations. They noted that muscle cross-sectional area and cortical bone cross-sectional area are diminished only in prepubertal T1DM individuals⁵ and that bone size normalizes with age⁷. Our findings are in concert with studies concerning bone in T1DM adults. Thrailkill K. et al.²¹ show reduced hip areal bone mineral density in men but not in women and Rakic V. et al.²² concluded that T1DM men but not women, may be at increased risk of osteoporosis.

Small methodological differences between the published studies exist. However, each study has its own reference group. In that, the possible impact of the methodological differences on the results seems to be mitigated. The limitation of the study is related to its cross-sectional design. Cross-sectional data could not necessarily reflect longitudinal changes in individual growth, sexual development and course of disease.

One of the new aspects of our study is its extensiveness. We studied all currently available pQCT outcomes in both measurement sites (4% and 66% of the forearm length), including bone mineral density, bone mass, cross-sectional dimension, longitudinal shape indexes, bone strength, muscle area and muscle/bone ratio.

It has been pointed out that T1DM girls showed a decreased ratio of cortical bone area to muscle area, which may suggest a lack of proper adaptation of the cortical bone to muscle force. Finally, T1DM adolescents in Tanner stage 5 diverged from younger individuals in terms of bone masses, dimensions and strength, which may suggest that bone shaft development in these individuals is impaired, affecting both size and strength.

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Acquisition of the data: EW, MS, MJ, PP.

Analysis of the data: MJ.

Interpretation of the data: MJ.

Revising of the work: MJ, EW, PP, MS.

All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors approved the final version of manuscript.

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