BONE SIZE NORMALIZES WITH AGE IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

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Running title: Bone development in children with Type 1 diabetes

Abbreviations: pQCT: peripheral quantitative computed tomography, CSA: cross-sectional area, BMD: bone mineral density, DEXA: Dual-Energy X-ray Absorptiometry, HbA1c: hemoglobin A1c, SD: standard deviation; ICT: intensified conventional therapy; CSII: continuous subcutaneous insulin infusion
Abstract

**Objective:** To establish whether type 1 diabetes mellitus (T1DM) has a long-term effect on bone development in children and adolescents.

**Methods:** Bone characteristics and muscle cross-sectional area (CSA) were analyzed cross-sectionally at study start in 41 (19f/22m) patients and reevaluated after 5.56 ± 0.4 years using peripheral quantitative computed tomography (pQCT). We hypothesize that bone size and muscle mass normalize with age.

**Results:** At first evaluation mean age was 9.87 ± 2.3 years and mean disease duration 4.31 ± 2.9 years. Height was -0.36 ± 1.9 SD and body mass index (BMI) was 0.39 ± 0.9 SD. Parameters of bone size were low in the whole patient group (corrected for patient’s height). At reevaluation, mean age was 15.44 ± 2.3 years and patients had a mean height of -0.12 ± 0.8 SD. BMI-SD had increased to 0.57 ± 1.1. Total and cortical CSA had normalized. Those patients with an increase in total CSA had a significant younger age at disease manifestation and a younger age at initial pQCT measurement. Bone size was well adapted to muscle mass expressed as the ratio of bone mineral content (BMC) per muscle mass and a close correlation was shown between rise in bone size and in muscle CSA (r=0.46, p=0.03).

**Conclusion:** Patients with manifestation of T1DM at an early age had transient impaired bone development. Within the follow-up period, the greatest increase in bone size was found in these patients. In adolescence, all patients had a normal bone size and an appropriate adaptation of bone on muscle.
Introduction
Studies on bone development in children with type 1 diabetes mellitus (T1DM) have generated conflicting results (1-5). There is still no conclusive data on the relative importance of several diabetes specific characteristics, such as age at onset, disease duration, and glycemic control or insulin regime, on bone health (6).

The majority of earlier studies are cross-sectional using dual-energy X-ray absorptiometry (DEXA) of the spine. Especially in pediatrics this method has limitations due to the two-dimensional measurement and therefore height dependency. Longitudinal data on relatively small numbers of patients over two to four years revealed a disturbed or normal bone development (7, 8). A recently published study over a wide time range from 12 to 84 months showed a slightly reduced mineralization of the spine independent of metabolic control or microvascular complications (9). The incidence of bone fractures was not increased in a large adult population with T1DM (10). Therefore, the clinical importance of possibly lower bone mineralization in T1DM is not clear.

The objectives of the present longitudinal study were to evaluate bone mineral density and bone size and muscle mass in patients with T1DM at two time points (5 ½ years apart) using peripheral quantitative computed tomography (pQCT). Interpretation of bone mineral density and geometry measurements is incomplete without taking into account muscle mass (11). Therefore we looked at the ratio between bone mineral content (BMC) and muscle mass (12).

Research Design and Methods
Subjects
In a cross-sectional study 88 Caucasian children and adolescents (42 females, 46 male) with T1DM were included. After a mean time of 5.56 ± 0.4 years 41 patients (20f/ 21m) could be reevaluated. Data analysis is restricted to the 41 patients with two pQCT measurements. All participants of the study were observed regularly at the diabetes outpatient clinic of the University Children's Hospital, Munich and met the following criteria: (a) first diagnosis of T1DM was made before 18 years of age, (b) no evidence of diabetic retinopathy, neuropathy or nephropathy, (c) no intake of medications, hormones, vitamins or calcium preparation in the preceding 6 months aside from insulin and if necessary thyroid hormones, (d) no chronic disease apart from positive thyroid antibodies with euthyroid status, (e) no hospitalization or ketoacidosis in the preceding 6 months and (f) no restriction of physical activity. All patients were examined every three months. At first measurement 38 patients had multiple injections (three to four daily) of regular and NPH insulin (ICT) and 3 had an insulin pump therapy (CSII). At second measurement 27 patients were on ICT and 14 patients on CSII. Diabetic control was monitored by measurements of hemoglobin A1c (HbA1c) levels at 3-months intervals. The HbA1c level was measured by DCA 2000™, based on specific inhibition of latex immunoagglutination (Bayer AG, Leverkusen, Germany). Normal values of HbA1c as established in our laboratory range from 4.0 % to 6.0%. Moreover, an average HbA1c was calculated for each patient taking the mean of four measurements during the previous 12 months before pQCT measurement.

Anthropometric data were compared with the cross-sectional German growth data of Kromeyer et al (13). The pQCT results were compared to those in a German reference population using identical methodology. The results of this reference population have been described before (14, 15, 16). Height was measured in a standing position to the next 1mm using a digital telescopic wall-mounted stadiometer (Ulmer Stadiometer, Prof. E. Heinze, Ulm, Germany). Weight was determined to the
nearest 0.1 kg using an electronic scale (Seca 753 E, Vogel and Hanke, Hamburg, Germany) with the children clothed in underwear. BMI was compared with the German normative data by Kromeyer et al (13). Forearm length was measured at the non-dominant forearm as the distance between the ulnar styloid process and the olecranon using a caliper. The stage of sexual development was determined in all study participants using the grading system by Tanner for breast development in girls and genital status in boys (17). Three Tanner stage groups were formed: prepubertal (Tanner stage 1), early pubertal (Tanner stage 2 and 3) and adolescent (Tanner stage 4 and 5).

Peripheral quantitative computed tomography
Two sites of the non-dominant radius were analyzed by pQCT, the distal metaphysis and the proximal diaphysis as previously described (14, 15, 16). In all patients, a pQCT scanner (XCT 2000, Stratec Inc.; Pforzheim, Germany) was used which is equipped with a low energy (38 keV) X-ray tube. The effective radiation dose is about 0.1 μSv from radiation source of 45 kV at 150 μA. For the measurement, the scanner was positioned on the distal forearm and a scout view was conducted to position the scanner at the site on the radius whose distance to the radial articular surface corresponded to 4% and 65% of the forearm length. At both sites a 2-mm-thick single tomographic slice was sampled at a voxel size of 0.4 mm. Image processing and calculation of numerical values were made using the manufacturer’s software package (version 5.40, Stratec Inc.; Pforzheim, Germany).

At the distal radius (metaphyseal site), total and trabecular BMD, at the proximal radius (diaphyseal site) total and cortical BMD, total CSA, cortical CSA, medullary CSA, muscle CSA, BMC and strength strain index (SSI), a measure of bone stability, were calculated by the manufacturer’s software.

Only measurements of good quality without movement artifacts were taken for analysis. To establish the variability of the measurements, the forearm of 6 adult volunteers was measured 3 times with repositioning of the forearm. Reproducibility was 1.08% for trabecular BMD and 1.42% for total BMD at the metaphysis and 1.30% for cross-sectional area, 1.36% for total BMD, and 1.11% for cortical BMD at the diaphysis. The accuracy of the previous version (XCT-960), not used by us, was determined using the European forearm phantom and average accuracy values between 1.9% and 1.4% for CSA and BMD values were reported (18).

Calibration of the machine was performed with phantoms provided by the manufacturer every other day (single slice) or once a month (multiple slices), respectively.

Statistical analysis
Results in T1DM patients were converted into sex-, age- and height-specific standard deviation scores (SD scores). To evaluate whether a parameter was significantly different from the results of an age-matched healthy population, the difference of the mean SD score to zero was assessed by Student’s two-tailed t test for unpaired observations or Mann-Whitney non-parametric U-test as appropriate. A significant difference was assumed when the 95% confidence interval of the mean SD score did not include zero. Pearson’s product-moment correlation was used to determine r values for possible influencing factors on bone mineral density and geometry parameters. We used a general univariate linear regression analysis (ANCOVA) to evaluate the covariant effects using significant levels of the 2-sided p-values < 0.05. We checked the normality of residuals and homoscedasticity. All statistical analyses were performed using the SPSS software package (version 14.0 for windows; SPSS Inc.; Chicago, USA).
Results

Auxologic and clinical data

Auxological and diabetes specific data are given in Table 1. At first evaluation diabetic patients showed reduced SD scores for mean height, which had normalized at second measurement. Significant higher mean SD scores for BMI were present at both time points, with a markedly higher number of female patients with a BMI-SD above 2 at second evaluation (5 females vs. 1 male). Most patients were in moderate to acceptable metabolic control with a range for average HbA1c of 5.8 – 9.2%, median 7.2%, at first pQCT and 6.2 – 11.0%, median 7.6%, at second pQCT measurement. Comparing our patients’ HbA1c levels within the DPV-data base, a German diabetes acquisition system for prospective surveillance, they were well within expected levels for patients with T1DM of this age (19). There was only a slight difference between latest and averaged HbA1c levels (Table 1). HbA1c values and daily insulin dose per kg body weight were similar in boys and girls. The older the patients and the longer the diabetes duration, the higher was the daily insulin dose per kg body weight (r=0.61, p<0.001), the present and averaged HbA1c (r=0.36 and 0.38, p=0.02, respectively) at first measurement. The form of insulin administration (ICT or CSII) had no influence on HbA1c or insulin dose. There were three patients with positive thyroid antibodies who were euthyroid without hormone replacement. All were well within the range for height, weight, BMI and HbA1c of all T1DM patients. No patient had celiac disease.

Bone Densitometric Results (Table 2)

At first measurement mean SD value of trabecular BMD was even higher in T1DM patients than in healthy controls, irrespective of age, sex and Tanner stage. Data analysis was performed using the new logarithmic approach as suggested by Rauch et al. (20). At the diaphysis, patients with type 1 diabetes had significantly reduced mean SD-values for total, cortical and medullary CSA as well as cortical BMD at first measurement. After 5.5 years, these parameters had normalized. Bone strength strain index (SSI) and muscle CSA were not significantly different from the reference population at first and second measurement. Bone size was well adapted to muscle mass expressed as the ratio of BMC per muscle CSA (figure).

Muscle CSA-SD values correlated significantly with total bone CSA (r=0.64 and r= 0.69, p<0.01) and cortical CSA (r=0.75 and r= 0.71, p<0.01) at first and second measurement, respectively. Separating the patients according to sex or Tanner stage (prepubertal, early pubertal or adolescent), there was no significant difference in BMD, bone size parameters or muscle CSA. However, at first measurement, the majority was prepubertal and at second measurement the majority was adolescent. Group numbers were too small to detect any difference for each Tanner stage. No difference between the sexes was found after correction for Tanner stages.

Influencing factors

In order to look for potential confounders, diaphyseal total bone and muscle CSA were used as dependent variables, latest HbA1c / average HbA1c, insulin dose and diabetes duration as the independent variables. Age, sex, pubertal stage and BMI were used as covariates. There was no significant influence of the independent variables on diaphyseal total and muscle CSA at both time points. There was no significant influence of insulin regime (ICT, CSII) on bone and muscle CSA parameters.

The younger the patients at disease manifestation and at first evaluation, the more increase in total CSA was detectable (r= -0.45 and r= -0.54, p<0.01, respectively). Additionally, the change in total cross-sectional area was significantly correlated with the change in muscle CSA (r=0.46, p=0.03). A linear regression model revealed age at manifestation (β=0.32, p=0.028) and change in muscle CSA (β=0.41, p=0.05) as
the major influencing factors for bone size development, accounting for 31% of variability (p=0.005). Muscle development was significantly influenced by the change in BMI-SD (β=0.32, p=0.025), the change in total CSA (β=0.4, p=0.006) and the change in average HbA1c (β=0.26, p=0.07), accounting for 39% of variability (p=0.01).

Discussion

Bone mineral density and bone mineral content have been measured cross-sectionally in children and adolescents with T1DM using different methodology. Most reports indicate a decreased bone mass in relation to a healthy reference population. Although, loss of bone mass has not been classified as a typical complication of T1DM, a relation to metabolic control has been proposed (21). Longitudinal studies on bone development in T1DM are sparse. In this investigation patients were studied after a fixed time interval of 5 to 6 years from first measurement. Therefore, a possible impact of disease manifestation on bone development should be excluded. Longitudinal data on skeletal growth and mineral acquisition are necessary to distinguish between temporal or long-term effects of diabetes on bone development. Previously we identified early manifestation of T1DM as a risk factor for smaller bone size and hypothesized, that there could be a normalization of bone size parameters with age (22). We further proposed that after clinical manifestation of T1DM an initial derangement of bone development may take place, possibly followed by a catch-up in bone development over a long period of time. In this study we could show that patients with an early onset of disease had the most pronounced increase in bone size over time. There was also a greater increase in muscle CSA, both accounting for approximately 31% of variability of change in total CSA-SD values. Gunczler et al (23) described a decreased BMD of the lumbar spine in children a few months after the onset of clinical T1DM. In a follow up study on patients with mean disease duration of 4.3 ± 2.9 years they reported on a stabilization of BMD parameters expressed as z-scores after one year (7). However, Moyer-Mileur et al (8) stated that in adolescents with mean disease duration of 4.2 ± 3.1 years gain of tibial cortical mineral bone was attenuated. A lower ratio of BMC/lean body mass was inversely related to HbA1c levels. After a period of more than 5 years after disease manifestation McNair et al. (24) noted a stabilization of BMD values. He suggested a defect in bone mass accretion early in the course of T1DM, which then ameliorates with time. This is in accordance with data from adult patients with a significant bone loss at time of diagnosis of T1DM and a possibly rapid decrease within the first year, followed by attainment of normal values (25, 26).

In analogy to our results, the effect of metabolic control on BMD and bone mass development has been excluded by several authors (6, 27). The HbA1c in our patient population was quite satisfactory and comparable with a German T1DM patient population of the same age (19). Therefore we cannot exclude the possibility that children with poorly controlled diabetes over a prolonged period of time might have more significant bone deficits than our patients. Further disease specific parameters such as insulin dose, insulin treatment regime or duration of disease had no significant influence on bone mineral density and bone size.

A significant increase in bone size took place in the majority of patients. This catch-up in bone cross-sectional size was most pronounced in those patients with early manifestation of T1DM at a young age. Possibly, patients with the diagnosis of T1DM early in life may be more susceptible to the metabolic derangement after clinical manifestation. Muscle CSA correlated significantly with total and cortical CSA levels, supporting the mechanostat theory in patients with T1DM and putting emphasis on the importance of the muscle-bone unit (11). Overall, muscle and bone development were well adapted indicated by a normal
ratio of BMC per muscle CSA. This is in contrast to the study by Moyer-Mileur et al (8) reporting an approximately 8% reduction of BMC for muscle CSA in T1DM adolescents. This discrepancy may result from the fact that their patients were younger and less advanced in puberty than ours.

Limitation of our study is the relatively small number of patients. Strength of our study is the long observation period and the comparison of mostly prepubertal children at first measurement with postpubertal adolescents at second measurement.

In conclusion, an early onset of T1DM may be a risk factor for transient altered bone CSA. Bone size normalizes over time and is well adapted to muscle size. Diabetes specific parameters seem to be less important for bone development. A longitudinal study beginning at diabetes manifestation could provide further insight in bone development in children and adolescents with T1DM.

Acknowledgement:
We thank Birgit Filipiak for statistical support.
References:


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<th></th>
<th>1&lt;sup&gt;st&lt;/sup&gt; measurement (n=41, 20 f, 21 m)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; measurement (n=41, 20f, 21m)</th>
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<tr>
<td>Age (yr)</td>
<td>9.87 ± 2.3</td>
<td>15.44 ± 2.3</td>
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<tr>
<td>Age at onset of disease (yr)</td>
<td>5.54 ± 3.2</td>
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<td>Disease duration (yr)</td>
<td>4.31 ± 2.9</td>
<td>9.81 ± 2.8</td>
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<tr>
<td>Height-SD</td>
<td>-0.36 ± 1.0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.12 ± 1.0</td>
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<td>Weight-SD</td>
<td>0.11 ± 1.1</td>
<td>0.46 ± 1.0</td>
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<tr>
<td>BMI-SD</td>
<td>0.39 ± 0.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.57 ± 1.0&lt;sup&gt;°&lt;/sup&gt;</td>
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<td>Tanner stage group (n=)</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>29</td>
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<tr>
<td>Latest HbA1c (%)</td>
<td>7.13 ± 0.9</td>
<td>8.14 ± 1.2&lt;sup&gt;°&lt;/sup&gt;</td>
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<tr>
<td>Average HbA1c (%)</td>
<td>7.11 ± 0.8</td>
<td>7.74 ± 1.1&lt;sup&gt;°&lt;/sup&gt;</td>
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<tr>
<td>Insulin dose (IU/kg/d)</td>
<td>0.78 ± 0.2</td>
<td>0.91 ± 0.2&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significant difference (p<0.05) for mean values between patients and reference population<sup>*</sup> and between the two measurement time points <sup>°</sup>;
Table 2. Longitudinal development of bone geometry, bone mineral density and muscle cross sectional area in patients with type 1 diabetes mellitus

<table>
<thead>
<tr>
<th>SD-values</th>
<th>1st measurement (n=41)</th>
<th>2nd measurement (n=41)</th>
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<tr>
<td>Metaphyseal</td>
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<tr>
<td>TD</td>
<td>0.28± 1.2</td>
<td>-0.01 ± 1.1</td>
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<tr>
<td>TrabD</td>
<td>0.45 ± 1.1*</td>
<td>-0.14 ± 1.1°</td>
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<tr>
<td>TCSA</td>
<td>-0.48 ± 0.9*</td>
<td>0.05 ± 1.0°</td>
</tr>
<tr>
<td>CCSA</td>
<td>-0.32 ± 1.0*</td>
<td>0.1 ± 1.0°</td>
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<tr>
<td>MCSA</td>
<td>-0.31 ± 0.8*</td>
<td>0.09 ± 1.0°</td>
</tr>
<tr>
<td>CTH</td>
<td>-0.11 ± 0.8</td>
<td>0.02 ± 0.8</td>
</tr>
<tr>
<td>relCCSA</td>
<td>-0.06 ± 0.8</td>
<td>0.01 ± 0.8</td>
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<tr>
<td>Diaphyseal</td>
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<td></td>
</tr>
<tr>
<td>TD</td>
<td>-0.09 ± 0.8</td>
<td>-0.06 ± 0.8°</td>
</tr>
<tr>
<td>CtD</td>
<td>-0.37 ± 1.0*</td>
<td>0.16 ± 0.9°</td>
</tr>
<tr>
<td>SSI</td>
<td>-0.26 ± 1.1</td>
<td>0.05 ± 1.0°</td>
</tr>
<tr>
<td>MsI-CSA</td>
<td>-0.24 ± 1.0</td>
<td>-0.05 ± 1.2°</td>
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<tr>
<td>BMC/MsI-CSA</td>
<td>-0.31 ± 0.9</td>
<td>0.19 ± 0.9</td>
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TrabD: trabecular bone mineral density; TD: total bone mineral density; TCSA: total bone cross sectional area; CCSA: cortical cross sectional area, MCSA: medullary cross sectional area, CtD: cortical bone mineral density, SSI: bone strength strain index; MsI-CSA: muscle cross sectional area; BMC: bone mineral content

Significant difference (p<0.05) for mean values between patients and reference population* and between the two measurement time points °.
Figure 1

[Graph showing data with different markers and lines indicating changes over age (years).]