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COMPLICATIONS

Childhood type 1 diabetes is associated with abnormal bone development

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Abstract

Objective: To describe bone mineral density (BMD), bone structure, and fracture prevalence in adolescents with type 1 diabetes (T1D) and explore their associations with glycemic control and microvascular complications.

Research design and methods: Cross sectional study of 64 adolescents (38 males) with T1D duration >10 years who underwent dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), fracture survey, plantar fascia thickness, and microvascular complications assessment.

Results: Mean age was 16.6 ± 2.1 years, diabetes duration 12.8 ± 2.2 years and HbA1c 8.9 ± 1.7% (74 mmol/mol). Fracture prevalence was 50%. DXA areal BMD (*Z*-score) was reduced for femoral neck (−0.5 ± 1.3, $p = 0.008$) and arm (−0.4 ± 1.0, $p < 0.001$), while total areal BMD and lumbar spine BMD were normal. In pQCT (*Z*-score), trabecular volumetric BMD (vBMD) was reduced for tibia (−0.4 ± 0.8, $p < 0.001$) and radius (−0.8 ± 1.4, $p < 0.001$) whereas cortical vBMD was increased at both sites (tibia: 0.5 ± 0.6, $p < 0.001$, radius: 0.7 ± 1.5, $p < 0.001$). Muscle cross-sectional area (CSA) was reduced for upper (−0.6 ± 1.2, $p < 0.001$) and lower (−0.4 ± 0.7, $p < 0.001$) limbs. DXA total areal BMD was positively correlated with BMI ($p < 0.01$) and age at T1D diagnosis ($p = 0.04$). Lower radial bone CSA, total and lumbar spine BMD were associated with autonomic nerve dysfunction. HbA1c, diabetes duration, fracture history and other microvascular complications were not significantly associated with bone parameters.

Conclusions: Adolescents with childhood-onset T1D have site-specific bone deficits in upper and lower limbs but normal total and lumbar spine BMD. T1D appears to have differential effects on trabecular and cortical bone compartments. Future longitudinal analysis is warranted to examine whether these changes translate in to increased fracture risk.

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KEYWORDS
adolescent, bone health, child, DXA, pQCT, type 1 diabetes

1 | INTRODUCTION

The association between type 1 diabetes (T1D) and vascular complications is well established, with evidence-based guidelines recommending early screening and management.[1] Our recent systematic review demonstrated abnormal bone development in youth with T1D, but highlighted the small number of studies with use of peripheral quantitative computed tomography (pQCT) and lack of use of bone mineral density (BMD) *Z*-scores.[2] Moreover, fracture prevalence was not examined.

Adults with T1D have greater fracture risk[3–6] with hip fracture occurring two decades earlier compared to those without T1D, resulting in significant disability to the individual and cost implications to the health care system.[3,5] As in the general population, low BMD is a risk factor for fractures in adults with T1D.[6] However, the fracture frequency observed in people with T1D is disproportionately greater than could be attributed to the reduction in BMD alone; suggesting poor bone quality may contribute to reduced bone strength.[6]

Dual-energy X-ray absorptiometry (DXA) is useful in determining bone mineral content (BMC), areal BMD and body composition. However, DXA is bone size dependent and does not provide true volumetric BMD (vBMD) nor bone quality parameters to determine bone strength. In contrast, pQCT provides information on vBMD (size independent); bone geometry (bone cross-sectional area [CSA], cortical thickness), from which determinants of strength can be made; muscle CSA and infer the functional muscle-bone unit (BMC/muscle CSA).[7] As T1D may affect bone compartments differently, pQCT has the added advantage of independently evaluating trabecular and cortical bone. Importantly, DXA and pQCT performed together provide complementary information on bone health.

DXA studies in adults with T1D have shown low total body, hip and lumbar spine BMD.[6,8,9] Results with DXA are less reproducible between pediatric studies due to its dependency on bone size, with some studies reporting low[10–20] whereas others report normal[21–27] total, lumbar spine and hip BMD. Only few pediatric studies have looked at pQCT parameters in children with T1D but the focus was on either radius[28–30] or tibia.[13,18,31] To our knowledge, only one pediatric study reported DXA, radial and tibial pQCT parameters; all three in the same study cohort[16] but the diabetes related characteristics including microvascular complications were not assessed.

We hypothesize that T1D negatively affects BMD and bone structure parameters in growing children and adolescents, leading to a higher risk of low trauma fracture. We also hypothesize that the presence of microvascular complications and suboptimal glycemic control are associated with adverse effects on bone parameters.

Hence, the primary aim of this study was to describe BMD and bone structure parameters using DXA and pQCT in adolescents with long duration T1D in comparison to population norms. The secondary aim was to assess fracture prevalence and physical activity in the study cohort and explore associations between BMD/bone structure, microvascular complications and indices of glycemic control.

1.1 | Methods

1.1.1 | Study design and population

This was a cross-sectional study of 64 (26 females, 38 males) adolescents with T1D who were regularly followed through the Diabetes Complications Assessment Service (DCAS) at The Children's Hospital at Westmead, Sydney, Australia. Inclusion criteria were age 11–20 years and T1D duration greater than 10 years. Exclusion criteria were presence of other chronic disease known to affect bone health such as osteogenesis imperfecta, severe physical disability (Gross Motor Function Classification System GMFCS >1), rickets within 1 year of study enrolment and previous bisphosphonate treatment. Participants were recruited between December 2016 and April 2018.

The study was approved by the Sydney Children's Hospitals Network Human Research Ethics Committee (reference number: HREC/16/SCHN/21). Informed consent was obtained from all participants and their carers prior to participation.

1.1.2 | Study visit and data collection/clinic assessment

Each study participant underwent standardized diabetes complications assessment with collection of demographic, clinical characteristics (including chronological age, age at T1D diagnosis, T1D duration, mode of insulin therapy (multiple daily injections—MDI, or continuous subcutaneous insulin infusion—CSII), total daily insulin dose (units/kg/day), presence of other autoimmune conditions, regular medications other than insulin) and anthropometric parameters (height, weight and body mass index [BMI]) with *Z*-scores computed using Centers for Disease Control and Prevention (CDC) 2000 reference data).[32]

Self-reported questionnaire on fracture history (site and age) and physical activity based on the Physical Activity Questionnaire for Adolescents (PAQ-A) by Kowlaski et al.[33] were collected for each participant. The PAQ-A questionnaire was modified to suit common Australian recreational physical activity as compared with typical Canadian recreational physical activity.[33]

1.1.3 | Bone densitometry

Total body (with arm and leg subregions), lumbar spine and right proximal femur BMD, and body composition parameters were assessed by

DXA (GE-Lunar iDXA, enCORE software v16.20) using manufacturer recommended acquisition and analysis techniques. The "extended" analysis option was used for the total body BMD. Lumbar spine bone mineral apparent density (BMAD) was calculated as per Carter et al.[34] to reduce the influence of height. Gender-specific age and height *Z*-scores were generated using in-house reference data reflective of healthy Australian children. A smaller subset of the in-house data have been previously published.[35] Since the original publication, the reference cohort has been expanded ($n > 500$), and the data reanalyzed to reflect changes due to software and machine upgrades. Prior to scanning the first participant of the day, quality assurance was performed using a proprietary calibration block. Additionally, a minimum of four per week quality control scans of an encapsulated aluminum spine phantom were performed. DXA BMD coefficient of variation (CV) of 341 lumbar spine scan performed during the study period was 0.2%.

pQCT scans of the non-dominant radius and tibia were performed using a Stratec XCT 2000 (proprietary software version 6.00B), 0.4 mm voxel and scan speeds of 15 and 20 mm/s, respectively. The non-dominant side for each participant was defined as which foot they would kick a ball with and the non-writing arm. If the participant reported a fracture of the non-dominant limb within the previous 2 years, the other side was measured ($n = 1$). The scan acquisition option to use limb length and a "reference" position to automatically determine the scan sites was utilized. Limb length was manually measured using a tape measure. The distance between the olecranon and the distal tip of the ulna styloid was used for radius length, and the distance between the superior margin of the medial condyle and the medial malleolus was used for tibial length. A "scout" scan was performed to determine the reference position. To minimize the potential impact of growth plates on the measurement, the chosen reference position was immediately distal to the most proximal sclerotic region surrounding the growth plate. The scans were performed at the 4% site of the tibia and radius, 65% site of the radius and 66% site of the tibia, where the "*X*% site" is defined as the distance *X*% of the limb length proximal to the "reference" position. Bone measurements included vBMD (mg/cm^3), trabecular vBMD, cortical vBMD, total and cortical CSA (mm^2), muscle CSA (mm^2), total and cortical BMC (mg/mm), and polar strength-strain index (pSSI, mm^3). Conversions from pQCT raw data to sex- and age-matched *Z*-scores were based on published pediatric reference data and personal correspondence (Rauch and Schoenau, and Moyer-Mileur).[36–38] We used the same pQCT protocol as the reference data. Quality assurance was performed using the proprietary "standard" phantom prior to scanning the first participant of the day. The pQCT "standard" phantom has a CV 0.3% for trabecular vBMD. A "cone" phantom was measured at least every 2 weeks.

Height *Z*-scores for DXA measures were used to adjust for any variance in stature within and between groups, while age *Z*-scores were used for pQCT as it is a true vBMD measure.

1.1.4 | Assessment of glycemic control

Glycemic control was assessed by HbA1c at the study visit and mean HbA1c from all available data since diagnosis of T1D. HbA1c was measured using high performance liquid chromatography (Diamat BioRad; non-diabetic range 4%–6%). Variation in HbA1c over the duration of diabetes was calculated as previously described.[39] For each participant, the intrapersonal mean and standard deviation (SD) of all recorded HbA1c were calculated, and SD-HbA1c was considered a measure of glycemic variability.[39] As the number of individual visits can influence the SD-HbA1c, values were divided by the number of visits to adjust for this possibility. Plantar fascia thickness, used as a surrogate marker of tissue glycation, was measured using ultrasound (LOGIQ-e Ultrasound, GE medical system) as previously described by our group.[40]

1.1.5 | Biochemical evaluation

A non-fasting blood sample was collected from each participant for C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), 25-hydroxy vitamin D (25OHD) and alkaline phosphatase [ALP]. Serum samples were kept frozen at −80°C and thawed once with immediate analysis after all the samples were collected. ALP and CRP were measured by the VITROS slides method using VITROS chemistry products (Ortho-Clinical Diagnostics, Inc.), 25OHD by liquid chromatography tandem mass spectrometry (LC/MS/MS) using Waters Xevo TQS (Waters Corporation, UK), and ESR by the Westergren method.

1.1.6 | Diabetes complications assessment

A comprehensive standardized diabetes complications assessment was performed at the study visit to assess for evidence of early retinopathy (seven fundal field stereoscopic retinal photography using the modified Airlie House Classification), nephropathy (three timed overnight urine collection for albumin excretion rate [AER] or the average of three early morning urine samples for albumin to creatinine ratio [ACR]), peripheral neuropathy (standardized biothesiometry) and autonomic neuropathy (heart rate variability), as previously described.[41–43] Early retinopathy was defined as the presence of at least one microaneurysm or one hemorrhage (grade 21) and early renal dysfunction as mean AER >7.5 μg/min or ACR ≥1 mg/min for male and ACR ≥1.4 mg/min for female.

1.1.7 | Sample size calculation and statistical analyses

A cohort of 64 participants was required to detect a difference of 0.5 SD at 5% alpha and greater than 80% in a normally distributed outcome (DXA and pQCT bone parameters) between adolescents with T1D and the population norm using a one-sample t-test. Descriptive statistics are reported as mean ± SD for normally distributed continuous variables and as median [IQR] for skewed continuous variables. Proportions are presented as number (%). Bone health measures (DXA and pQCT) in study participants were compared to population norms

TABLE 1 Participant demographics and clinical characteristics, $n = 64$

Participant characteristics	Mean ± SD or number (percentage)
Females/males	26 (41%)/38 (59%)
Age at visit (years)	16.6 ± 2.1
Age at type 1 diabetes diagnosis (years)	3.8 ± 2.2
Type 1 diabetes duration (years)	12.8 ± 2.2
HbA1c at visit % (mmol/mol)	8.9 ± 1.7% (74 mmol/mol)
Mean HbA1c cumulative over diabetes lifespan % (mmol/mol)	8.4 ± 0.9% (68 mmol/mol)
HbA1c variability (SD-HbA1C)	0.86 ± 0.36
Plantar fascia thickness (mm)	0.20 ± 0.03
Total daily insulin dose (units/kg/day)	0.96 ± 0.24
CSII/MDI (number of patients)	51 (80%)/12 (20%)
Height *Z*-score	0.11 ± 0.9
Weight *Z*-score	0.67 ± 1.0*
BMI *Z*-score	0.62 ± 1.1*
Overweight/obese	11 (17%)/11 (17%)

Note: *p value <0.001 compared to CDC 2000 reference population.[32]
Abbreviations: BMI, body mass index; CSII, continuous subcutaneous insulin infusion; HbA1c, glycated hemoglobin A1c; MDI, multiple daily insulin injection; SD, standard deviation.

using one-sample t tests. Different groups within the study population were compared using two-sample t tests. Associations between bone parameters and various normally distributed diabetes-related characteristics were examined using Pearson correlations. Spearman rank correlations were used to examine associations between physical activity and bone parameters. Forward stepwise linear regression models were used to examine associations between bone parameters (DXA and pQCT), diabetes complications, HbA1c, diabetes duration, age and BMI. Data analysis was performed using IBM SPSS software (version 24, SPSS Inc.).

1.2 | Results

1.2.1 | Population characteristics

The study comprised 64 adolescents (38 males, 59%) with a mean age of 16.6 ± 2.1 years at the study visit (Table 1). The participants were primarily Caucasian (94%) and similar to the reference population used for bone parameter comparison. Twenty-two (12 males and 10 females) adolescents (34%) were classified as overweight or obese (BMI SD >1). Thirteen (20%) adolescents had other well-controlled autoimmune conditions: 7 (11%) with coeliac disease, 3 (4.7%) with hypothyroidism, 3 (4.7%) with hypothyroidism and coeliac disease, and 1 (1.6%) with coeliac disease and Graves' disease. The majority of adolescents had achieved their final height (75%) with the remainder at varying puberty stages. Microvascular complication rates were as follows: autonomic nerve dysfunction—45% (29/63); early renal dysfunction—30% (19/57); peripheral nerve dysfunction (abnormal temperature and/or vibration sensation)—25% (16/64); and early non-proliferative retinopathy—23% (15/61). Median 25OHD[27] was 63 nmol/L; 16/63 (25%) had insufficiency (30–50 nmol/L) and 4/63 (6%) deficiency (<30 nmol/L). Two participants had mildly elevated ALP compared to age and sex specific reference range. Inflammatory markers (CRP and/or ESR) were elevated in 12/63 (19%) participants.

1.2.2 | Bone parameters and diabetes

Dual-energy X-ray absorptiometry

There were no significant differences in total BMD, BMC, bone area and lumbar spine BMAD *Z*-scores between study participants and the reference population (Table 2). Participants had significantly lower right femoral neck ($p = 0.008$) and arm BMD ($p = 0.001$) *Z*-scores. 17/64 (27%) adolescents had right femoral neck BMD *Z*-score ≤ −1.5. Participants also had significantly higher fat percentage ($p < 0.001$) and higher lean tissue mass (LTM) ($p = 0.045$).

Peripheral quantitative computed tomography

Tibial and Radius pQCT demonstrated significantly lower trabecular vBMD at the 4% site and higher cortical vBMD at the 66% site compared to the reference population (Table 2). Radial cortical parameters at the 65% site were not significantly different whereas tibial cortical bone parameters including cortical BMC, CSA and thickness were significantly lower than the reference data.

Muscle CSA in both upper and lower limbs was significantly lower than the reference data. Cortical BMC adjusted for muscle CSA was normal. Some publications report muscle CSA adjusted for either height or limb length. As our cohort had normal height and no significant correlation was found between limb length and muscle CSA age *Z*-scores, further adjustment of muscle CSA was deemed unnecessary.

1.2.3 | Bone densitometry, pQCT and diabetes related characteristics

Higher BMI and older age were positively correlated with total BMD *Z*-score (Pearson correlation $r = 0.45$, $p < 0.01$ and $r = 0.46$, $p < 0.01$, respectively) (Figure 1). Age at T1D diagnosis had a weak positive correlation with total BMD *Z*-score (Pearson correlation $r = 0.26$, $p = 0.04$). However, no significant correlations were identified between diabetes duration, mean HbA1c (current or cumulative over diabetes life span) or glycemic variability (SD-HbA1c) and total BMD *Z*-score or other DXA and pQCT bone parameters.

1.2.4 | Bone densitometry, pQCT and microvascular complications

In both uni- and multivariable regression models, autonomic nerve dysfunction was associated with lower total BMD and lumbar spine

TABLE 2 Dual-energy X-ray absorptiometry (DXA) (height adjusted *Z*-scores) and peripheral quantitative computed tomography (pQCT) (age adjusted *Z*-scores) bone parameters in T1D adolescents in comparison to healthy population reference data, $n = 64$

DXA parameters			pQCT parameters					
	Height *Z*-score			Age *Z*-score			Age *Z*-score	
	Mean ± SD	*p* value	Radius	Mean ± SD	*p* value	Tibia	Mean ± SD	*p* value
Total BMD	−0.1 ± 1.2	0.57	4% Total BMC	**−1.0 ± 1.3**	**<0.001**	*	*	*
Total BMC	0.1 ± 1.4	0.64	4% Trabecular vBMD	**−0.8 ± 1.4**	**<0.001**	4% Trabecular vBMD	**−0.4 ± 0.8**	**<0.001**
Bone area	0.1 ± 1.0	0.61	4% Total bone CSA	**−0.7 ± 1.1**	**<0.001**	*	*	*
L1–4 BMAD	−0.2 ± 1.0	0.055	65% Cortical BMC	−0.1 ± 1.0	0.54	66% Cortical BMC	**−0.4 ± 0.9**	**0.001**
Right femoral neck BMD	**−0.5 ± 1.3**	**0.008**	65% Cortical vBMD	**0.7 ± 1.5**	**<0.001**	66% Cortical vBMD	**0.5 ± 0.6**	**<0.001**
Arms BMD	**−0.4 ± 1.0**	**0.001**	65% Cortical CSA	−0.2 ± 1.0	0.18	66% Cortical CSA	**−0.5 ± 0.9**	**<0.001**
			65% Cortical thickness	−0.2 ± 1.0	0.15	66% Cortical thickness	**−0.8 ± 0.8**	**<0.001**
Fat % (age *Z*-score)	**0.9 ± 1.2**	**<0.001**	65% pSSI	−0.1 ± 1.0	0.57	66% pSSI	**0.6 ± 1.0**	**<0.001**
Lean tissue mass	0.4 ± 1.6	0.045	Muscle CSA	**−0.6 ± 1.2**	**<0.001**	Muscle CSA	**−0.4 ± 0.7**	**<0.001**
BMC for LTM	−0.3 ± 1.2	0.11	*	*	*	Cortical BMC/muscle CSA	0.0 ± 0.9	0.89

Note: *p* value refers to differences from healthy population reference data.[35–38] Bold values represent statistically significant results.
Abbreviations: *, reference data not available; BMC, bone mineral content; BMD, areal bone mineral density; CSA, cross-sectional area; DXA, dual energy X-ray absorptiometry; LTM, lean tissue mass; pQCT, peripheral quantitative computed tomography; SD, standard deviation; vBMD, volumetric bone mineral density.

BMAD on DXA (Table 3). It was also associated with lower radial total CSA and tibial cortical thickness on pQCT. Other microvascular complications were not significantly associated with DXA or pQCT parameters.

1.2.5 | Physical activity and fracture survey results

Sixty-two participants completed the survey. Thirty-one (50%) adolescents (23 males, 8 females) reported sustaining at least one fracture, and seven (22%) of them had sustained >1 fracture since the diagnosis of T1D. Males (62%) reported fractures more than females (32%) ($p = 0.019$). The forearm was the commonest fracture site followed by the lower leg. There was no association between positive fracture history and any of the bone parameters by DXA or pQCT. The physical activity survey (modified PAQ-A) median summary score was 1.8 (1.2) (where a score of 1 indicates low and 5 indicates high physical activity), suggesting a low physical activity level in study participants compared to healthy children.[44] Self-reported median daily screen time was 360(240) min, significantly higher than the recommended health guideline of <120 min/day[45] but similar to healthy Australian teenage children.[46] There was no association between activity level and muscle or bone parameters.

2 | DISCUSSION

To our knowledge, this is the first study to comprehensively assess bone health in adolescents with T1D and its association with microvascular complications. Although there was no evidence of osteoporosis in our cohort, we found a statistically significant reduction in right femoral neck BMD, arm BMD, trabecular vBMD and total and cortical CSA, along with higher cortical vBMD compared to normative reference data. This may adversely affect peak bone mass accrual and strength, resulting in the well described increased susceptibility to fracture later in life.[3] We also found lower muscle mass in both upper and lower limbs, raising the possibility of diabetic myopathy having adverse effects on bone development.[47]

Lower radial and tibial trabecular vBMD was evident on pQCT as was the right femoral neck BMD, which also reflects trabecular structure in the hip. However, DXA lumbar spine BMAD, which mainly reflects the axial trabecular compartment, was normal. This may reflect the differential growth pattern of the axial and appendicular skeleton, where axial growth mainly occurs during the pubertal years and appendicular growth in the pre-pubertal years. All study participants had T1D onset before the onset of puberty and therefore any ill effects of disease may affect appendicular bones more than the axial bones. The contrasting results may also reflect the inherent difference between the way bone is evaluated by DXA and pQCT. For example, in pQCT, metaphyseal trabecular bone can be measured separately from cortical bone, whereas while spine DXA predominantly measures trabecular bone, it also measures the cortical shell of the vertebrae.

Multiple studies have examined bone parameters in children with T1D by DXA and have reported normal[22–26] or low lumbar spine BMD,[10,12–17,19,20] but these studies had heterogeneous study populations, limiting direct comparison between them, as highlighted in our recent systematic review.[2] Our study cohort had lower right femoral neck BMD similar to previous studies in young adults.[15,23,25] Indeed, 27% of study participants had right femoral neck BMD *Z*-score ≤ −1.5, which is clinically relevant and is consistent with increased hip fracture risk in adults with T1D.[3,5] To date, only eight

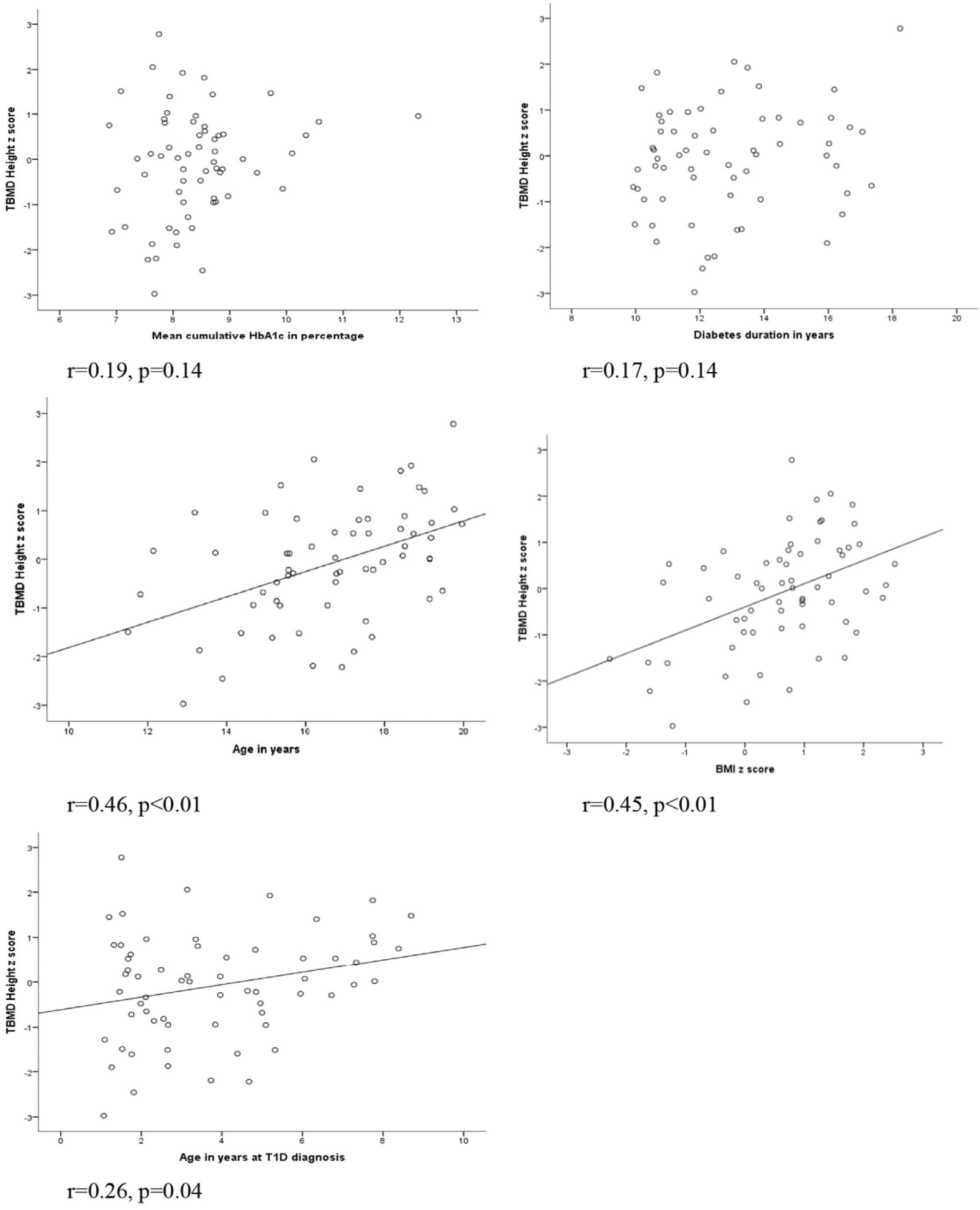

FIGURE 1 Associations between total bone mineral density and various clinical characteristics, $n = 64$.

studies have been published with pQCT findings in pediatric T1D; four studies measured radius,[28–30,48] three studies tibia[13,18,31] and only one study measured both sites.[16] The majority of studies showed reduction in trabecular vBMD,[13,16,18,48] similar to our study, with few exceptions.[28,30] One study showed increased trabecular vBMD in pre-pubertal children with T1D at baseline with significant reduction

TABLE 3 Uni- and multivariable linear regression models exploring associations between bone parameters and various diabetes-related characteristics

	Univariable models		Multivariable models	
Outcome and factors	***B* (95% CI)**	***p* value**	***B* (95% CI)**	***p* value**
Total BMD *Z*-score (DXA)				
Diabetes duration	−0.05	0.72	–	
HbA1c	0.02	0.85	–	
BMI *Z*-score	0.43 (0.17, 0.55)	<0.001	**0.43 (0.17, 0.54)**	**<0.001**
Autonomic nerve dysfunction	−0.31 (−1.13, −0.14)	0.01	**−0.31 (−1.06, −0.18)**	**0.007**
Lumbar spine BMAD *Z*-score (DXA)				
Diabetes duration	−0.05	0.69	–	
HbA1c	0.04	0.78	–	
BMI *Z*-score	0.32 (0.08, 0.55)	0.01	**0.32 (0.09, 0.54)**	**0.008**
Autonomic nerve dysfunction	−0.31 (−1.30, −0.15)	0.02	**−0.31 (−1.27, −0.17)**	**0.01**
Radial 65% cortical CSA *Z*-score (pQCT)				
Diabetes duration	−0.16	0.21	–	
HbA1c	−0.13	0.30	–	
BMI *Z*-score	0.33 (0.08, 0.55)	0.008	**0.32 (0.09, 0.53)**	**0.007**
Autonomic nerve dysfunction	−0.33 (−1.33, −0.20)	0.009	**−0.32 (−1.28, −0.21)**	**0.007**
Radial 65% total CSA *Z*-score (pQCT)				
Diabetes duration	−0.11	0.39	–	
HbA1c	0.009	0.95	–	
BMI *Z*-score	0.31 (0.06, 0.49)	0.01	**0.30 (0.06, 0.48)**	**0.01**
Autonomic nerve dysfunction	−0.26 (−1.1, −0.02)	0.04	**−0.25 (−1.07, −0.03)**	**0.04**
Tibia 66% cortical thickness *Z*-score (pQCT)				
Diabetes duration	0.12	0.33	–	
HbA1c	−0.12	0.34	–	
BMI *Z*-score	0.35 (0.08, 0.44)	0.004	**0.35 (0.09, 0.43)**	**0.004**
Autonomic nerve dysfunction	−0.30 (−0.97, −0.09)	0.02	**−0.29 (−0.93, −0.10)**	**0.02**

Abbreviations: –, no association found; BMAD, bone mineral apparent density; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CSA, cross-sectional area; DXA, dual energy X-ray absorptiometry; HbA1c, glycated hemoglobin A1c; pQCT, peripheral quantitative computed tomography. Bold values represent statistically significant results.

after 5 years,[29,30] suggesting longer duration T1D, as in our cohort, may lead to a reduction in trabecular values.

Interestingly, we observed increased radial and tibial cortical vBMD in our cohort, similar to findings by Maratova et al.[31] and Moyer-Mileur et al.[13] This finding, in conjunction with low trabecular vBMD, suggests increased material bone density, which reflects an increase in mineralization. This has been described in osteogenesis imperfecta type 1, where the hypothesis is that more "space" between type 1 collagen allows for hydroxyapatite crystal deposition,[49] and also in prolonged immobility, a low bone turnover state, where older bone has more time to mineralize and there is a reduction in Haversian canals.[50] We postulate that, the increased material density in T1D may be due to reduction in bone turnover or an abnormal collagen glycosylation, which may allow for an increase in mineral deposition.

We observed a reduction in total and cortical CSA, similar to other studies.[13,16,28,30] Our study population also had reduced muscle CSA in both upper and lower limbs, similar to the study by Bechtold et al.[30] Interestingly, we found higher LTM on DXA, possibly due to an artefactual rise from central and visceral adiposity. However, BMC for LTM and cortical BMC adjusted for muscle CSA were normal in our cohort, showing appropriate response of bones to muscle mass and importance of the "muscle-bone unit".

Another study reported significant reduction in muscle function, with reduced muscle force and power, in adolescents with T1D.[31] Our study cohort was physically less active than their healthy counterparts. However, multiple other studies have shown no difference in the activity level between adolescents with T1D and their healthy peers.[13,30,51] Therefore, altered bone and muscle findings may not simply be explained by the level of physical activity. Some studies have proposed a direct effect of diabetes on muscles by altered protein and energy metabolism, altered hormonal milieu and motor nerve axonal loss.[52] Muscle mass and function are important factors for bone development, maintenance, modeling and remodeling.[47]

Therefore, reduction in muscle mass and/or function in T1D may contribute to adverse bone development in addition to any primary bone effects.

The complications assessments were performed to detect early signs of microvascular complications and may explain relatively higher rates seen in our study cohort with long diabetes duration. Our study demonstrates novel associations between microvascular complications and various DXA and pQCT parameters in adolescents with T1D after adjusting for disease duration or glycemic control. In our cohort, autonomic nerve dysfunction was associated with lower radial total CSA bone. In addition, autonomic nerve dysfunction was also associated with lower total BMD, lumbar spine BMAD and lower tibial cortical thickness. Although there are no pediatric studies looking at the effect of microvascular complications on bone parameters, multiple adult studies have demonstrated higher fracture risk in the presence of nephropathy, neuropathy and retinopathy.[3,53] Two prospective adult studies have shown reduced femoral neck areal BMD[54] and progressive bone loss with reduced BMC[55] in T1D group with retinopathy and neuropathy compared to T1D group without complications despite similar glycemic control or duration of disease. More recent study by Shanbhogue et al. also reported deficits in cortical and trabecular bone vBMD and microarchitecture in a T1D population with microvascular disease regardless of disease duration or glycemic control.[56]

The fracture prevalence in our study group was higher than in previous studies in adolescents with T1D.[13,16,18] However, lifetime risk of fracture in childhood is approximately 42%–64% in males and 27%–40% in females,[57] consistent with fracture prevalence in our cohort. Future research looking at fracture rates and physical activity in children with diabetes compared to their healthy peers in a larger study are necessary to further our understanding in this area.

3 | STRENGTHS

Strengths of our study include the comprehensive assessment of bones by use of pQCT, DXA, and a fracture and physical activity survey, and the ability to systematically evaluate microvascular complications to explore associations between bone health and other complications. Our study adds to the body of information available on pediatric diabetes osteopathy.

4 | LIMITATIONS

The cross-sectional design enables only associations to be explored but not causality. Longitudinal studies with different duration of diabetes will be important. We did not specifically evaluate dietary calcium intake. However, all children with T1D at our centre receive dietary assessment and advice for optimum calcium intake at a regular interval. The lack of matched control group is a limitation of our study. However, the reference population ($n > 500$) data used to compare the DXA parameters in our study were derived from local population and had similar ethnicity to the study cohort. We do not have local reference data for pQCT parameters and hence published pediatric references were used for comparison. However, we used the same pQCT protocols as the published references and the study cohort was of similar ethnicity. To our knowledge, there are no reference data available for DXA and pQCT (4%/65% or 66% radius and tibia) both in the same population. Our pQCT results showed similar changes in the trabecular and cortical bone parameters as described in previous studies with control population[13,18,31] and adds to the information available on bone quality parameters in youth with T1D. We acknowledge that our study was likely underpowered to explore associations between microvascular complications and bone parameters.

5 | CONCLUSION

Adolescents with pre-pubertal onset of T1D and greater than 10 years diabetes duration showed overall normal total BMD and lumbar Spine BMD. However, they demonstrated site-specific bone deficits in upper and lower limb both with low femoral neck and arm BMD as well as lower bone CSA and muscle mass. Younger age at T1D diagnosis and autonomic neuropathy may contribute to such bone deficits. T1D seems to have differential effects on trabecular and cortical bone compartments suggesting pQCT as a useful adjunct to DXA in the assessment of diabetes related bone changes. Future longitudinal studies are needed to determine if these changes translate into increased fracture risk and to delineate underlying pathogenesis to help devise prevention strategies.

AUTHOR CONTRIBUTIONS

Komal A. Vora contributed to study design, ethics application, recruitment, data collection, statistical analyses, data interpretation, and wrote the manuscript. Craig F. Munns contributed to study design, ethics application, data interpretation, and revised the manuscript. Kim C. Donaghue contributed to study design and revised the manuscript. Julie Briody contributed to data collection, data interpretation and revised the manuscript. Maria E. Craig contributed to study design and revised the manuscript. Paul Benitez-Aguirre conceived the study design, and contributed to ethics application, recruitment, data collection, statistical analyses, data interpretation, and revised the manuscript and is the guarantor of this article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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