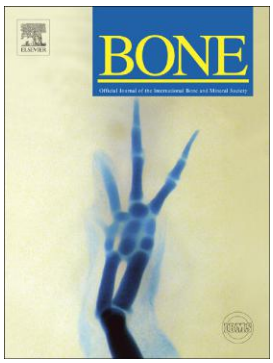

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Adolescents with long-duration type 1 diabetes have low bone mass and reduced levels of bone indices reflecting altered bone resorption

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ABSTRACT

The prevalence of type 1 diabetes (T1D) is increasing globally and is associated with severe complications, including an increased risk of fractures. This case-control study investigated whether young individuals with well-controlled, long-duration T1D have differences in bone mass and bone biomarkers in comparison with healthy matched controls. Fifty individuals, aged 15.0-17.9 years, with a T1D duration of at least 8 years and (mean±SD) 10.6±2.1 years were included, hence the participants had diabetes throughout most part of their puberty and growth spurt. The mean HbA1c since diabetes diagnosis was 56±6 mmol/mol (7.3±0.6%). Bone mass was assessed by dual-energy X-ray absorptiometry and peripheral quantitative computed tomography (pQCT). Clinical follow-up data were retrieved from the Swedish National Diabetes Registry. The control group comprised 50 healthy matched adolescents, aged 15.1-17.9 years. The groups were well-matched with no significant differences in age, sex, weight, height, body mass index and the self-reported physical activity. Total body less head aBMD and Z-scores were significantly lower in T1D individuals, $p<0.05$. Total tibia density and trabecular density, by pQCT, were also lower in the T1D group, $p<0.05$. There were no differences between the groups for parathyroid hormone, 25-hydroxyvitamin D, bone-specific alkaline phosphatase (BALP), intact procollagen type I N-propeptide (PINP), sclerostin, bioactive sclerostin and osteoprotegerin. However, individuals with T1D had reduced levels of C-terminal telopeptide of type I collagen (CTX) ($p<0.001$) and nuclear factor κB ligand (a.k.a. RANKL) ($p=0.01$), indicating altered regulation of osteoclasts. In conclusion, young individuals with well-controlled, long-duration T1D have subnormal bone mass accrual, impaired microstructure at several sites and suppressed RANKL-mediated osteoclastogenesis resulting in reduced bone resorption. Based on these findings, we suggest that bone health should be monitored in pediatric diabetes care to potentially intervene early in life in susceptible individuals to achieve optimal peak bone mass.

Key words: Bone mineral density; bone biomarkers; DXA; forearm; pediatric

Abbreviations

25(OH)D, 25-hydroxyvitamin D

aBMD, areal bone mineral density

BMC, bone mineral content

BALP, bone-specific alkaline phosphatase

BMU, bone multicellular units

CTX, C-terminal telopeptide of type I collagen

CV, coefficient of variation

DXA, dual-energy X-ray absorptiometry

DXL, dual-energy X-ray absorptiometry and laser

ELISA, enzyme-linked immunosorbent assay

NDR, Swedish National Diabetes Registry

OPG, osteoprotegerin

PINP, intact procollagen type I N-propeptide

PTH, parathyroid hormone

pQCT, peripheral quantitative computed tomography

RANKL, receptor activator of nuclear factor κB ligand

T1D, type 1 diabetes

T2D, type 2 diabetes

TBLH, total body less head

1. Introduction

Type 1 diabetes (T1D) is increasing globally [1] and is the second most prevalent chronic disease among children in Sweden. T1D is a complex disease that affected approximately 8.4 million individuals (95% uncertainty interval 8.1–8.8 million individuals) worldwide in 2021, of whom 1.5 million (18%) were younger than 20 years of age [2]. At present, individuals diagnosed with T1D have no cure to anticipate and must rely on the quality of their self-care and medical care to alleviate and prevent acute and long-term complications [3]. Over the past 100 years, the treatment with insulin injections has become increasingly effective, as well as the use of HbA1c and other biomarkers for metabolic regulation [4]. To achieve an optimal insulin replacement, support from advanced technologies such as sensors and pumps have been available in recent decades improving the metabolic control [5-7].

Glycemic disturbances due to diabetes can affect multiple organs and cause severe complications of body tissues [8]. Micro- and macrovascular complications of T1D, such as retinopathy, nephropathy, neuropathy and cardiovascular disease, are well-documented in the scientific literature [9,10]. Over the past decade, there has been increasing awareness of bone health and how T1D negatively impacts bone tissue. There appears to be a potential link between fracture risk and glycemic control, but the underlying pathophysiological mechanisms are complex and may also include accumulation of advanced glycation end-products in bone tissue [11-13]. Disease duration, at least in less well-regulated T1D, is an important factor contributing to the development of diabetes-related complications. Many factors influence bone health and bone accrual in young individuals who have not yet reached their peak bone mass, including genetics, mechanical loading, physical activity, longitudinal growth, medications, as well as endocrine and nutritional factors [13].

Multiple studies have shown that individuals with T1D, as well as type 2 diabetes (T2D), have an increased risk of fractures [14-16]. A cohort study based on a Swedish

population of adults with T1D (*n* = 24,605) concluded that both females and males have an increased risk of hip fracture [17]. In the systematic review by Janghorbani et al., [15] it was shown that the association between hip fractures was stronger for T1D than for T2D, and that both females and males have an increased risk. In addition, dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) have demonstrated reduced bone mass in children with T1D. According to a meta-analysis, young individuals with T1D have lower bone mineral content (BMC), areal bone mineral density (aBMD) and deficits in trabecular density and skeletal microarchitecture [18].

While conclusive data has been published regarding reduced aBMD and increased fracture risk among individuals with T1D, investigations with bone biomarkers reflecting bone remodeling show a large heterogeneity with inconsistent results in T1D studies [19-22]. Most studies have found low levels of C-terminal telopeptide of type I collagen (CTX) and low or normal bone formation markers in T1D in comparison with healthy individuals. Data on bone regulatory molecules such as sclerostin, osteoprotegerin (OPG) and receptor activator of nuclear factor κB ligand (RANKL) are inconclusive [21-23]. Hence, there are several knowledge gaps related to bone biomarkers reflecting mechanisms regulating bone remodeling in adolescents with T1D that warrant further investigation.

In this case-control study, we hypothesized that young adolescents with long-duration diabetes (more than 8 years), have altered bone parameters and biochemical biomarkers in comparison with healthy matched controls, despite a well-regulated T1D. The aim was to investigate bone mass and an extensive panel of bone biomarkers in adolescents with a narrow age span and long-duration T1D (throughout the pubertal period), in comparison with a matched healthy control group.

2. Materials and methods

2.1. Subjects and study design

Fifty adolescents, aged 15.0–17.9 years, comprising 23 females and 27 males, with T1D duration of at least 8 years (mean ± SD, 10.6 ± 2.1 years; range 8.0–16.5 years), were included in this case-control study (Fig. 1). All individuals with T1D were identified from the Swedish National Diabetes Registry (NDR) and were followed regularly at the Queen Silvia Children's Hospital in Gothenburg, Sweden. All pediatric clinics (n = 43) in Sweden prospectively report data from every clinical follow-up visit to the NDR, which includes 97.5% of all children aged 0–17.9 years.

The control group comprised 50 age- and sex-matched healthy adolescents (aged 15.1–17.9 years, 22 females and 28 males) living in the surrounding Gothenburg area. The control individuals were enrolled continuously over the study period. They were relatives and friends to the individuals with T1D, and adolescents related to hospital staff. Control individuals were not physically examined, and no clinical routine blood samples were taken. The research staff met each control individual and went through the questionnaire and exclusion criteria. The study commenced in April 2019 and was completed in June 2022. Clinical data for all individuals are presented in Table 1. Exclusion criteria included celiac disease, obesity, hypothyroidism, metabolic, skeletal and inflammatory diseases, breastfeeding and pregnancy.

The study was approved by the regional research ethics committee of the University of Gothenburg (no. 1076-18) and was conducted according to the 1964 Helsinki declaration and its later amendments. All participants and caregivers received written and oral information prior to study enrollment, and written consent was obtained from each study participant and their caregivers.

2.2. Height and weight measurements

Height was measured using a wall-mounted ruler to the nearest 0.1 cm (Ulmer stadiometer, Prof. Heinze, Ulm, Germany), and weight was measured on electronic scales to the nearest 0.1 kg. Z-scores were calculated for height, weight and BMI in reference to Swedish pediatric data sets [24,25].

2.3. Registry data

Clinical follow-up data for the T1D individuals were retrieved from the NDR registry. The duration of diabetes was calculated as time between the date of diabetes diagnosis and the study date when the DXA scan was performed. For each individual, the most recent HbA1c value, the mean (SD) and median (minimum; maximum) HbA1c of all the entries in the diabetes registry since the diabetes diagnosis (excluding the first three months), as well as HbA1c during three age intervals (0–8.9 years, 9.0–13.9 years and 14.0–17.9 years) were calculated to account for the glycemic control during different growth periods. Other clinical data retrieved from the T1D diagnosis to the study end included insulin treatment regimen (pen or pump), use of glucose sensor, total daily dose of insulin (units per kg body weight), body weight, height, BMI-SDS, (calculated according to Swedish population reference data), the most recent blood pressure value, and status concerning microalbuminuria and retinopathy.

2.4. Questionnaires

Questionnaires were completed by the participants in privacy during their study visit, with a research team member present to answer any potential questions. The questionnaire included questions regarding insulin treatment, current medications, use of supplements, previous

fractures, and tobacco usage. Physical activity during the previous week was reported using a validated self-assessment form, i.e., the Swedish version of the International Physical Activity Questionnaire [26].

2.5. Assessment of bone mass

All measurements were performed at Queen Silvia Children's Hospital, Gothenburg, Sweden. Total body aBMD was assessed by DXA, Lunar iDXA (GE Lunar Corp., Madison, WI, USA). Calculated Z-scores by Lunar are age- and sex-specific. DXA was also used to assess fat and lean body mass. The intraindividual coefficient of variation (CV) was 0.5% for total body BMD and 0.7% for lumbar spine [27].

The measurements with pQCT were performed on the left tibia at 4% and 66% of the tibia length using the XCT 2000 (Stratec Medizintechnik GmbH, Pforzheim, Germany), software version 6.00. By bone microstructures, we are referring to measurements of trabecular and cortical compartments by pQCT. Quality control calibration was performed using a standard protocol and every 30 days using a cone phantom protocol and calibration tools/phantom were provided by the manufacturer. The tibial length was measured with a plastic ruler from the medial malleolus to the medial tibial plateau. A scan speed of 20 mm/s and a voxel size of 0.5 mm were used. The position of the CT scans was defined in a coronal scout scan at the foot ankle joint and to reduce noise, the image was filtered using a median filter before the analysis. The performance of the device has been reported elsewhere [28]. The polar strength strain index (SSI) of the bone cortex, which represents an estimation of the mechanical strength of cortical bone in the measured tibia, was calculated by the software [29]. CVs for the pQCT measurements were 0.4–1.3% [30].

The dual-energy X-ray absorptiometry and laser (DXL) Calscan system (Demetech AB, Täby, Sweden) was used to measure left calcaneal aBMD as reported elsewhere [31]. CVs for the DXL measurements were 0.8–1.4%.

2.6. Biochemical determinations

Blood samples (non-fasting, because of their diabetes) were collected from all study participants and aliquots were stored at –80°C until analysis. Serum intact parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) were analyzed at the Department of Clinical Chemistry (Swedac accredited no. 1342), Linköping University Hospital, Sweden, and all samples were assayed with reagents from the same batch. Intact PTH was determined with the Elecsys electrochemiluminescence immunoassay on a Roche Cobas e601 platform (Roche Diagnostics Scandinavia AB, Gothenburg, Sweden), with an assay performance of: analytical range 0.13–530 pmol/L, and total CV of ≤7%. Serum 25(OH)D was measured on a LIAISON® XL analyzer with the total 25(OH)D chemiluminescence immunoassay (DiaSorin, Stillwater, MN, USA), which demonstrates 100% cross-reactivity for 25(OH)D_2 and 25(OH)D_3. This 25(OH)D method has an assay performance of: analytical range 10–375 nmol/L, and total CV of ≤8%.

Intact procollagen type I N-propeptide (PINP) was assessed with the UniQ radioimmunoassay (Aidian Oy, Espoo, Finland), with an assay performance of: analytical range 5–250 µg/L, intra-assay CV of <5%, and interassay CV of <6%. Serum BALP was measured by the MicroVue™ BAP enzyme-linked immunosorbent assay (ELISA) (Quidel Corp., San Diego, CA), with an assay performance of: analytical range 0.7–140 U/L, intra-assay CV of <6%, and interassay CV of <8%. CTX was assessed in EDTA plasma samples with the CrossLaps® ELISA (Immunodiagnostic Systems Holdings Ltd.), with an assay

performance of: analytical range 20–3380 ng/L, intra-assay CV of <6%, and interassay CV of <10%.

Serum sclerostin and bioactive sclerostin were measured by quantitative ELISA (Biomedica, Vienna, Austria). The sclerostin assay (BI-20492) had an assay performance of: analytical range 3.2–240 pmol/L, intra-assay CV of <7%, and interassay CV of <10%. The bioactive sclerostin assay (BI-20472) had an assay performance of: analytical range 1.9–320 pmol/L, intra-assay CV of ≤2%, and interassay CV of ≤5%. Serum OPG and free soluble RANKL were assayed by ELISA. The OPG assay (BI-20403, Biomedica) had an assay performance of: analytical range 0.07–20 pmol/L, intra-assay CV of ≤3%, and interassay CV of ≤5%. The RANKL assay (BI-20462, Biomedica) had an assay performance of: analytical range 0.01–2.00 pmol/L, intra-assay CV of ≤3%, and interassay CV of ≤5%.

2.7. Statistical analysis

Difference between groups were identified using the Mann-Whitney test. Correlations were assessed by Spearman's rank correlation coefficient. *P*-values of less than 0.05 were considered significant. Power calculations were made regarding total body aBMD less head. To detect a difference of SD 0.14 with the power of 90% using the Mann-Whitney test, with the significance level of 0.05, a sample of 42 individuals was needed in each group. All statistical analyses were performed using R software (v. 4.2.3; https://www.r-project.org/; The R Project, Vienna, Austria). The R packages ggplot2 and ggpubr were used for visualization and to add significance levels to the plots.

3. Results

Clinical data and characteristics of individuals with T1D and healthy matched controls are presented in Tables 1 and 2. The groups were well-matched with no significant differences

regarding age, sex, weight, height, BMI, self-reported physical activity and geography. Fourteen individuals with T1D had sustained 16 fractures, and 18 control subjects had 19 fractures before study start (Table 1). No vertebral fractures were observed among the study participants. Only a few individuals were smokers or used snuff tobacco (Table 1). Even though there was no statistical difference in BMI between the T1D group and controls, the T1D group had a higher total fat mass, $p < 0.05$. No differences were observed between the groups for total lean mass measured by DXA.

3.1. Bone mass parameters

Total body less head (TBLH) aBMD and TBLH Z-score were lower in T1D individuals in comparison with the control group (Table 3). There were no differences between the groups for lumbar spine L_1-L_4 aBMD and Z-score. Femur aBMD and Z-score were lower in the T1D group, $p = 0.014$ and $p = 0.008$, respectively. Radius ultra distal aBMD was lower in the T1D group in comparison with the control group, $p = 0.028$. Ulna ultra distal aBMD was lower in the T1D group in comparison with the control group, $p = 0.005$. From the pQCT measurements, it was found that total tibia density and trabecular density were lower in the T1D group in comparison with the control group, $p < 0.05$ (Table 4). Trabecular aBMD at the calcaneus, the most peripheral site, was significantly lower in the T1D group (Table 3).

3.2. Bone biomarkers

Serum levels of 25(OH)D were (mean ± SD; minimum – maximum) 65 ± 20; 18 – 105 nmol/L and 67 ± 14; 31 – 102 nmol/L, in T1D and controls, $p = 0.79$. Two individuals with T1D had 25(OH)D levels below 25 nmol/L (18 nmol/L and 24 nmol/L). These samples were taken in January; however, both these individuals had PTH levels within the reference

interval. Serum PTH levels were 3.6 ± 1.7; 1.3 – 10.3 pmol/L and 3.8 ± 1.4; 1.2 – 8.8 pmol/L, in T1D and controls, respectively, and no difference was found between the groups, $p = 0.12$.

Results for the bone biomarkers PINP, BALP, RANKL, CTX, OPG, sclerostin and bioactive sclerostin are presented in Table 5..

Significant negative correlations were found between CTX and age for T1D (r = –0.33, $p = 0.02$) and controls (r = –0.36, $p = 0.01$). No significant correlation was found between RANKL and age for T1D (r = –0.25, $p = 0.08$), but significant for controls (r = –0.37, $p = 0.008$) (Fig. 2).

3.3. HbA1c in relation to bone markers and bone mass

None of the HbA1c measures in the study (HbA1c last measure, HbA1c mean 0-8 years, HbA1c mean 9-13 years, HbA1c mean 14-17 years, HbA1c mean 0-17 years and HbA1c mean last year) showed any significant correlation with any of the bone markers or DXA measurements. No significant correlations were found between HbA1c (groups as above) and the pQCT measurements (i.e., total tibia density, trabecular density, cortical density and cortical thickness).

3.4. Diabetes duration in relation to bone mass and bone markers

No associations were found between diabetes duration and bone mass parameters measured by DXA and pQCT. The levels of PINP (r = 0.33; $p = 0.021$), BALP (r = 0.41; $p = 0.003$) and CTX (r = 0.30; $p = 0.032$) were significantly correlated with diabetes duration, while PTH, 25(OH)D, OPG, RANKL, sclerostin and bioactive sclerostin showed no significant correlation.

3.5. Exercise

All participants answered the physical activity questionnaire. There was no difference regarding the amount of self-reported exercise performed between the T1D and control groups 623 ± 593 min/week and 503 ± 313 min/week ($p = 0.64$), respectively. Neither did we find any significant difference regarding the reported sedentary time between the T1D and control groups, 500 ± 131 min/day and 496 ± 154 min/day ($p = 0.56$), respectively.

4. Discussion

Adolescence is a sensitive period of bone acquisition that encompasses both modeling and remodeling to achieve an individual's maximal peak bone mass. This case-control study investigated adolescents with a long T1D duration (mean 10.6 years), alongside with a well-matched control group in terms of age, sex, weight, height, BMI, self-reported physical activity and geographical location, which resulted in a homogeneous study group. Despite well-controlled diabetes for most adolescents throughout the most part of the pubertal growth period, the T1D group exhibited impaired bone structural changes at several sites. TBLH aBMD and Z-scores were significantly lower in T1D individuals. Total tibia density and trabecular density, by pQCT, were also lower in the T1D group. There were no differences between the groups for PTH, 25(OH)D, BALP, PINP, sclerostin, bioactive sclerostin and OPG. Levels of CTX and RANKL were lower in the T1D group, suggesting altered osteoclast regulation.

Data in the current study shows that young individuals with T1D have lower TBLH aBMD and Z-score values. Bone mass parameters were also reduced at peripheral skeleton sites such as the ultradistal radius and ulna aBMD (non-loading site), and calcaneus aBMD (a site of loading) in comparison with the control group. As demonstrated by the pQCT measurements, no differences were found between the groups in terms of bone size

parameters, i.e., cortical thickness, endosteal circumference, periosteal circumference and SSI. Trabecular density was lower in T1D individuals, which coincides with the DXL data, as the calcaneus is predominately a site of trabecular bone [31]. This contrasts with the findings of Novak et al., [32] where the cortical bone strength (i.e., SSI) was lower in the T1D group. However, that group had a higher mean age (24 years), longer diabetes duration (mean 19 years) and less well-controlled diabetes in comparison to this study.

Microvascular complications are commonly observed early in T1D, [8] affecting tissues in peripheral sites, such as the feet. These microvascular alterations in T1D could impact the microstructure of the calcaneus bone, resulting in reduced bone mass in the trabecular bone at this peripheral site. It has been demonstrated that diabetes microvascular disease is associated with deficits in both cortical and trabecular volumetric BMD, and that changes in microarchitecture could partially account for the increased skeletal fragility previously found in adults with T1D (mean age 45 years) [33]. Walle et al., [34] showed that T1D results in impaired bone microstructure, fewer trabeculae and lower trabecular BMD. In the meta-analysis by Zheng et al., [18] it was concluded that children and adolescents (≤18 years) with T1D had deficits in trabecular density and skeletal microarchitecture.

According to Roggen et al., [35] who investigated individuals aged 17–19 years, those with T1D are at risk of achieving smaller bone size at the distal radius. This was predominantly observed in girls with increased adiposity. Our findings indicate less affected bone tissues in individuals with T1D at the 33% radius and ulna, which is a site with more cortical bone than the ultradistal radius and ulna (Table 3). As suggested by the 2019 International Society for Clinical Densitometry official position paper, [36] forearm DXA assessments are valuable for monitoring bone mass in diseases that affect bone health. The current study confirms this assertion. Our findings of normal lumbar spine bone mass are

consistent with another report comprising 44 children and adolescents with a disease duration of 6.6 years [37].

In line with our results, a meta-analysis summarized that CTX was significantly lower among individuals with diabetes [38]. The question arises whether the number of bone multicellular units (BMU), [39] which include both osteoclasts and osteoblasts, is reduced in adolescents with T1D. Although we found lower mean levels of the bone formation markers PINP and BALP in the T1D group, these markers did not significantly differ between the groups, which might reflect that the number of BMUs is not affected.

Several systematic reviews have presented that bone formation markers are lower in individuals with diabetes [38]. However, most studies included in these systematic reviews present data from adults with T2D and do not distinguish between T1D from T2D. Systematic reviews focusing specifically on children and adolescents with T1D show varied results for markers of bone formation, with lower or normal levels for PINP and BALP [38]. The interpretation of bone biomarkers in children and adolescents is challenging due to skeletal growth, bone accrual, and the onset of puberty during these developmental years.

The discovered association between the bone markers CTX, PINP and BALP, with the duration of T1D, but not with HbA1c, suggests that the impaired bone material properties and microarchitecture are primarily a consequence of the disease mechanisms per se in this group of individuals with well-controlled T1D. However, this does not exclude the potential influence of poor metabolic control on bone health.

Downregulation of RANKL leads to decreased osteoclast recruitment, resulting in fewer resorption pits and subsequently smaller volumes of bone tissue that could be replaced with new bone tissue. Conflicting results have been reported about RANKL and OPG in individuals with T1D. A 2021 meta-analysis reported higher levels of OPG, but concluded that strong evidence is missing because of the marked heterogeneity in a limited number of

well-designed studies [40]. In this study, we found lower levels of RANKL in the T1D group and OPG levels in the same magnitude as the control group, which results in a lower RANKL/OPG ratio in the T1D group. A reduced RANKL/OPG ratio inhibits osteoclastogenesis, which is in line with the significantly lower CTX levels found in the current study. Considering our findings with lower levels of RANKL and CTX, antiresorptive treatment with the RANKL-blocking monoclonal antibody denosumab may not be the first choice for individuals with low bone mass and T1D due to the low levels of RANKL.

The circulating levels of sclerostin and bioactive sclerostin were not different between healthy controls and adolescents with T1D, which suggests that pathways involving osteocytes were not negatively influenced in this cohort, which is in line with others using the same sclerostin immunoassay [41]. A 2017 meta-analysis reported increased sclerostin levels in both T1D and T2D [23]. The conflicting results regarding sclerostin could in part be due to that different sclerostin assays have been applied in different studies. Available sclerostin assays differ considerably due to detection of different antigenic epitopes, assay format and the fact that different antibodies are used [42]. We used two different assays for measuring sclerostin: the bioactive sclerostin is a second generation immunoassay for the intact sclerostin molecule, whereas the sclerostin assay also detects circulating fragments of sclerostin [42].

In relation to a previous study on skeletal health of young individuals with T1D in Sweden, [32] we may speculate that bone changes may happen in different stages of bone acquisition while attaining peak bone mass. Trabecular bone is affected initially and with longer diabetes duration the cortical bone may also become affected. The true chronological pathogenesis can, however, only be confirmed with future studies on the same young population with T1D.

There was no significant difference between T1D and controls regarding self-reported sedentary time or exercise pattern. This is contrary to studies showing that adolescents with T1D are more inactive and sedentary than apparently healthy peers [43-45]. In a well-controlled homogenous T1D group, like the current study, the chance of finding pathological bone results related to physical activity is low.

This study has several strengths, such as a well-matched control group in terms of age, sex, weight, height, BMI, self-reported physical activity and geographical location. It also includes validated data from a national pediatric diabetes register. Another strength is the long duration of T1D, on average 10.6 years from diagnosis, in a cohort of young individuals with most of their pubertal timespan influenced by T1D. Strict exclusion criteria were applied; individuals with obesity and other autoimmune diseases commonly found in individuals with T1D were excluded. Furthermore, two- and three-dimensional bone measurement techniques by DXA and pQCT were used in combination with measurements of bone markers reflecting formation, resorption, and regulatory molecules of osteogenesis. The T1D group was well-controlled with a mean HbA1c value of 56 mmol/mol or 7.3% (NGSP) since diabetes diagnosis, considering that adolescence is a challenging period for achieving good metabolic control. No difference in exercise patterns was found between T1D and controls. However, this study also has some limitations. Although a well-matched control group was used, adolescents were recruited from a single study center, and it was a cross-sectional study design. Examination of Tanner staging was not included in the study protocol; however, the mean age for both groups was 16.3 years, which suggests that the majority of the adolescent individuals were already in late puberty or past puberty.

In conclusion, young individuals with well-controlled long-duration T1D have lower TBLH aBMD and Z-score values. Furthermore, the data also demonstrated reduced tibia trabecular density by pQCT and altered bone mass measured by DXA in the radius and femur.

These findings indicate subnormal bone mass accrual and impaired bone structural changes at several sites. The reduced levels of RANKL and CTX indicate suppressed osteoclastogenesis, reduced bone resorption, and consequently smaller volumes of bone tissue that could be replaced with new bone tissue. Typically, adolescence is a period of bone acquisition that includes both modeling and remodeling to achieve an individual's maximum peak bone mass. Taken together, these results suggest that T1D is characterized by low bone mass and suppressed RANKL-mediated osteoclastogenesis, a consequence that becomes evident already at an early age. The findings of this study advocate for that bone health should be monitored in individuals with T1D to potentially intervene early in life in susceptible individuals to achieve optimal peak bone mass.

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CRediT authorship contribution statement

Diana Swolin-Eide: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing.

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Declaration of competing interest

The authors have no conflicts of interest associated with the material presented in this paper.

Data availability

Data presented in this study are available on request.

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Table 1. Clinical data of subjects with T1D and matched healthy controls

	T1D (*n* = 50)	**Controls** (*n* = 50)	***P*-value**
Age (years)	16.3 (0.9) 16.3 (15.0; 17.9)	16.3 (0.9) 16.2 (15.1; 17.9)	0.97
Weight (kg)	68.6 (11.8) 66.9 (50.0; 111.0)	66.5 (8.9) 67.6 (49.0; 84.4)	0.64
Weight (Z-score)	0.7 (1.0) 0.7 (–1.5; 3.3)	0.5 (1.1) 0.5 (–1.8; 2.6)	0.41
Height (m)	1.74 (0.09) 1.74 (1.56; 1.95)	1.74 (0.09) 1.74 (1.46; 1.93)	0.47
Height (Z-score)	0.2 (1.1) 0.0 (–2.0; 2.3)	0.3 (1.1) 0.5 (–3.6; 2.5)	0.37
BMI (kg/m^2)	22.7 (2.8) 22.1 (17.5; 30.3)	21.9 (2.7) 21.4 (17.0; 29.9)	0.14
BMI (Z-score)	0.7 (0.9) 0.6 (–1.5; 2.6)	0.4 (1.0) 0.4 (–1.5; 2.5)	0.13
Comorbidities	Allergy: pollen, mite (*n* = 4) Asthma (*n* = 1) Psoriasis (*n* = 1) ADHD (*n* = 1) Hypertension (*n* = 1) Autism, ADD (*n* = 1)	Allergy: pollen, mite, horse (*n* = 5) Eczema (*n* = 1) Asthma (*n* = 1) ADD (*n* = 1)	NS
Medications (other than insulin)	*n* = 14 (28%) Asthma/allergy (*n* = 3) Oral contraceptives (*n* = 4) Central stimulants (*n* = 3) Enalapril (*n* = 2) Acne medication (*n* = 1) Melatonin (*n* = 1) Atarax (*n* = 1) Antidepressant (*n* = 1)	*n* = 13 (26%) Allergy (*n* = 5) Oral contraceptives (*n* = 4) Central stimulants (*n* = 1) Acne medication (*n* = 1) Local steroid (*n* = 1) Antidepressant (*n* = 1)	NS
Supplements	16	12	0.50
Fractures	16 fractures in 14 individuals (28%): *forearm×8, rib, toe×2, humerus, ankle, wrist and finger×2. Plus 5 greenstick fractures: forearm×5	19 fractures in 18 individuals (36%): *forearm×6, rib, toe×2, hand, clavicle, tibia×2, foot, wrist×2, and finger×3. Plus 6 greenstick fractures: forearm×3, wrist and finger×2	0.68
Smoking (self-reported)	0	2	
Snuff tobacco	1	3	

T1D, type 1 diabetes. For categorical variables, n (%) is presented.
For continuous variables, mean (SD); median (minimum; maximum) are presented.
*Forearm includes radius, ulna and elbow.

Table 2. Clinical data and biochemical assessments of subjects with T1D

	T1D (*n* = 50)
Age at diabetes onset (years)	5.7 (2.4); 5.9 (0.9; 9.5)
Diabetes duration (years)	10.6 (2.1); 10.4 (8.0; 16.5)
Use of sensor (last visit)	49 of 50 (98%)
Insulin pump (last visit)	42 of 50 (84%)
Insulin (Units per kg body weight) (*n* = 43)	0.9 (0.2); 0.9 (0.6; 1.5)
Time in tight target (%), last visit (*n* = 40) (3.9-7.8 mmol/L; 70-120 mg/dL)	39 (11); 40 (20; 61)
Last HbA1c measurement (*n* = 50) (IFCC mmol/mol) / NGSP (%)	54 (9); 52 (37; 81) 7.1 (0.8); 6.9 (5.5; 9.6)
HbA1c since diabetes diagnosis (*n* = 50) (IFCC mmol/mol) / NGSP (%)	56 (6); 56 (41; 75) 7.3 (0.6); 7.3 (5.9; 9.0)
HbA1c, 0–8.9 years (*n* = 41) (IFCC mmol/mol) / NGSP (%)	55 (7); 56 (39; 71) 7.2 (0.6); 7.3 (5.7; 8.6)
HbA1c, 9.0–13.9 years (*n* = 48) (IFCC mmol/mol) / NGSP (%)	56 (7); 56 (41; 77) 7.3 (0.6); 7.3 (5.9; 9.2)
HbA1c, 14.0–17.9 years (*n* = 50) (IFCC mmol/mol) / NGSP (%)	56 (9); 55 (38; 82) 7.3 (0.8); 7.2 (5.6; 9.7)
Microalbuminuria	2 of 50 (4.1%)
Retinopathy (simplex)	3 of 50 (6.0%)
Systolic blood pressure (mmHg)	114 (9); 113 (99; 138)
Diastolic blood pressure (mmHg)	67 (7); 66 (49; 80)
High blood pressure (≥130/80 mmHg)	2 of 48 (4.2%)

For categorical variables, n (%) and for continuous variables, mean (SD) / median (minimum; maximum) are presented. Abbreviations: HbA1c, glycated hemoglobin A1c; IFCC, International Federation of Clinical Chemistry; NGSP, National Glycohemoglobin Standardization Program; T1D, type 1 diabetes. Continuous glucose monitoring, real time or intermittent scanning.

Table 3. Analyses of DXA and DXL in subjects with T1D and matched healthy controls

	T1D (*n* = 50)	**Controls** (*n* = 50)	***P*-value**
DXA measurements			
TBLH aBMD (g/cm^2) TBLH aBMD (g/cm^2)	1.01 (0.12) 0.99 (0.74; 1.35)	1.05 (0.11) 1.05 (0.83;1.30)	**0.039**
TBLH BMD Z-score	0.18 (0.95) 0.20 (–1.80; 2.70)	0.54 (0.86) 0.50 (–1.40; 2.30)	**0.036**
Lumbar spine (L_1-L_4) aBMD (g/cm^2) Lumbar spine (L_1-L_4) aBMD (g/cm^2)	1.16 (0.14) 1.16 (0.78; 1.46)	1.17 (0.13) 1.18 (0.89; 1.45)	0.617
Lumbar spine Z-score	–0.08 (1.05) –0.10 (–2.40; 2.10)	0.06 (0.93) –0.10 (–2.20; 1.80)	0.605
Total left femur aBMD (g/cm^2) Total left femur aBMD (g/cm^2)	1.04 (0.15) 1.01 (0.68; 1.39)	1.13 (0.15) 1.11 (0.82; 1.48)	**0.014**
Total left femur Z-score (g/cm^2) Total left femur Z-score (g/cm^2)	0.00 (1.14) –0.05 (–2.70; 2.70)	0.62 (0.97) 0.60 (–1.40; 2.80)	**0.008**
Radius aBMD Ultra distal (g/cm^2) Radius aBMD Ultra distal (g/cm^2)	0.41 (0.06) 0.40 (0.24; 0.56)	0.43 (0.06) 0.42 (0.28; 0.58)	**0.028**
Ulna aBMD Ultra distal (g/cm^2) Ulna aBMD Ultra distal (g/cm^2)	0.31 (0.05) 0.30 (0.23; 0.47)	0.34 (0.06), 0.34 (0.22; 0.48)	**0.005**
Radius aBMD 33 % (g/cm^2) Radius aBMD 33 % (g/cm^2)	0.81 (0.08) 0.80 (0.68; 1.00)	0.83 (0.08) 0.83 (0.66; 1.10)	0.210
Total fat mass (kg) Total fat mass (kg)	18.6 (9.2) 16.7 (6.2; 63.6)	14.9 (6.7) 12.7 (4.6; 29.8)	**0.027**
Total lean mass (kg) Total lean mass (kg)	44.1 (9.9) 41.4 (27.7; 71.2)	44.8 (8.1) 43.8 (29.7; 59.5)	0.471
DXL measurements			
Calcaneal aBMD (g/cm^2) Calcaneal aBMD (g/cm^2)	0.41 (0.08) 0.39 (0.28; 0.66)	0.45 (0.08) 0.45 (0.28; 0.60)	**0.007**

For continuous variables, mean (SD) / median (minimum; maximum) are presented. *P*-values are from Mann-Whitney. Bold indicates significant p-values. Abbreviations: aBMD, areal bone mineral density; DXA, dual-energy X-ray absorptiometry; DXL, dual-energy X-ray absorptiometry and laser; T1D, type 1 diabetes; TBLH, Total body less head.

Table 4. Analyses of pQCT in subjects with T1D and matched healthy controls

	T1D (n = 50)	**Controls** (n = 49)	***P*-value**
Total area (mm^2)	1183 (196) 1162 (864; 1714)	1221 (209) 1226 (760; 1588)	0.255
Total tibia density (mg/cm^3)	297 (41) 283 (224; 406)	312 (37) 312 (237; 387)	**0.012**
Trabecular density (mg/cm^3)	241 (36) 229 (166; 340)	255 (32) 255 (183; 308)	**0.020**
Cortical density (mg/cm^3)	1107 (34) 1101 (1012; 1173)	1092 (44) 1097 (990; 1174)	0.115
SSI (mm^3)	2244 (531) 2162 (1184; 3364)	2335 (579) 2333 (1190; 3630)	0.431
Total area (bone area) (mm^2)	688 (124) 677 (438; 923)	725 (133) 706 (468; 1082)	0.227
Cortical area (bone area) (mm^2)	299 (57) 284 (192; 421)	318 (51) 313 (219; 415)	0.053
Endosteal circumference 66% (mm)	62 (8) 61 (46; 82)	63 (9) 61 (48; 87)	0.622
Periosteal circumference 66% (mm)	87 (8) 87 (67; 102)	89 (8) 88 (72; 112)	0.260
Cortical thickness 66% (mm)	4.03 (0.62) 3.99 (3.00; 5.60)	4.20 (0.59) 4.06 (2.99; 5.71)	0.177

For continuous variables, mean (SD) / median (minimum; maximum) are presented.
P-values are from Mann-Whitney. Abbreviations: pQCT, peripheral quantitative computed tomography; SSI, strength strain index; T1D, type 1 diabetes.

Table 5. Bone biomarkers in subjects with T1D and matched healthy controls

	T1D (*n* = 50)	**Controls** (*n* = 50)	***P*-value**
25(OH)D (nmol/L)	65 (20) 64 (18; 105)	67 (14) 67 (31; 102)	0.791
PTH (pmol/L)	3.6 (1.7) 3.1 (1.3; 10.3)	3.8 (1.4) 3.5 (1.2; 8.8)	0.120
PINP (µg/L)	289 (204) 246 (51; 996)	412 (332) 321 (70; 1278)	0.140
BALP (U/L)	65 (46) 51 (21; 219)	74 (58) 56 (13; 256)	0.702
CTX (ng/L)	914 (507) 776 (216; 2046)	3347 (2380) 2603 (276; 10296)	**0.001**
OPG (pmol/L)	2.7 (0.8) 2.7 (0.9; 4.8)	2.6 (0.8) 2.5 (0.6; 4.3)	0.637
RANKL (pmol/L)	0.22 (0.14) 0.19 (0.01; 0.65)	0.32 (0.18) 0.29 (0.04; 0.79)	**0.011**
Sclerostin (pmol/L)	22.3 (8.8) 22.2 (7.5; 53.6)	22.5 (9.2) 20.9 (7.3; 44.3)	0.888
Bioactive sclerostin (pmol/L)	26.4 (9.6) 23.8 (12.5; 47.5)	27.7 (15.1) 24.7 (8.9; 101.2)	0.923

For continuous variables, mean (SD) / median (minimum; maximum) are presented.
P-values are from Mann-Whitney. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BALP, bone-specific alkaline phosphatase; CTX, C-terminal telopeptide of type I collagen; OPG, osteoprotegerin; PINP, intact procollagen type I N-propeptide; PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor κB ligand; T1D, type 1 diabetes.

Figure legends

Fig. 1. Flow chart of inclusion of individuals with T1D.

Fig. 2. CTX and RANKL scatterplots in relation to age for T1D and controls.

Significant negative Spearman's rank correlation coefficients were found between CTX and age for T1D (r = –0.33, p = 0.02) and for the control group (r = –0.36, p = 0.01). No significant correlation was found between RANKL and age for T1D (r = –0.25, p = 0.08), but significant for the control group (r = –0.37, p = 0.008).

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Type 1 diabetes (T1D) is associated with an increased risk of fractures
- Long-duration T1D results in low bone mass accrual and impaired microstructure
- Levels of RANKL and CTX were lower in the T1D group
- Bone health should be monitored in adolescents with T1D

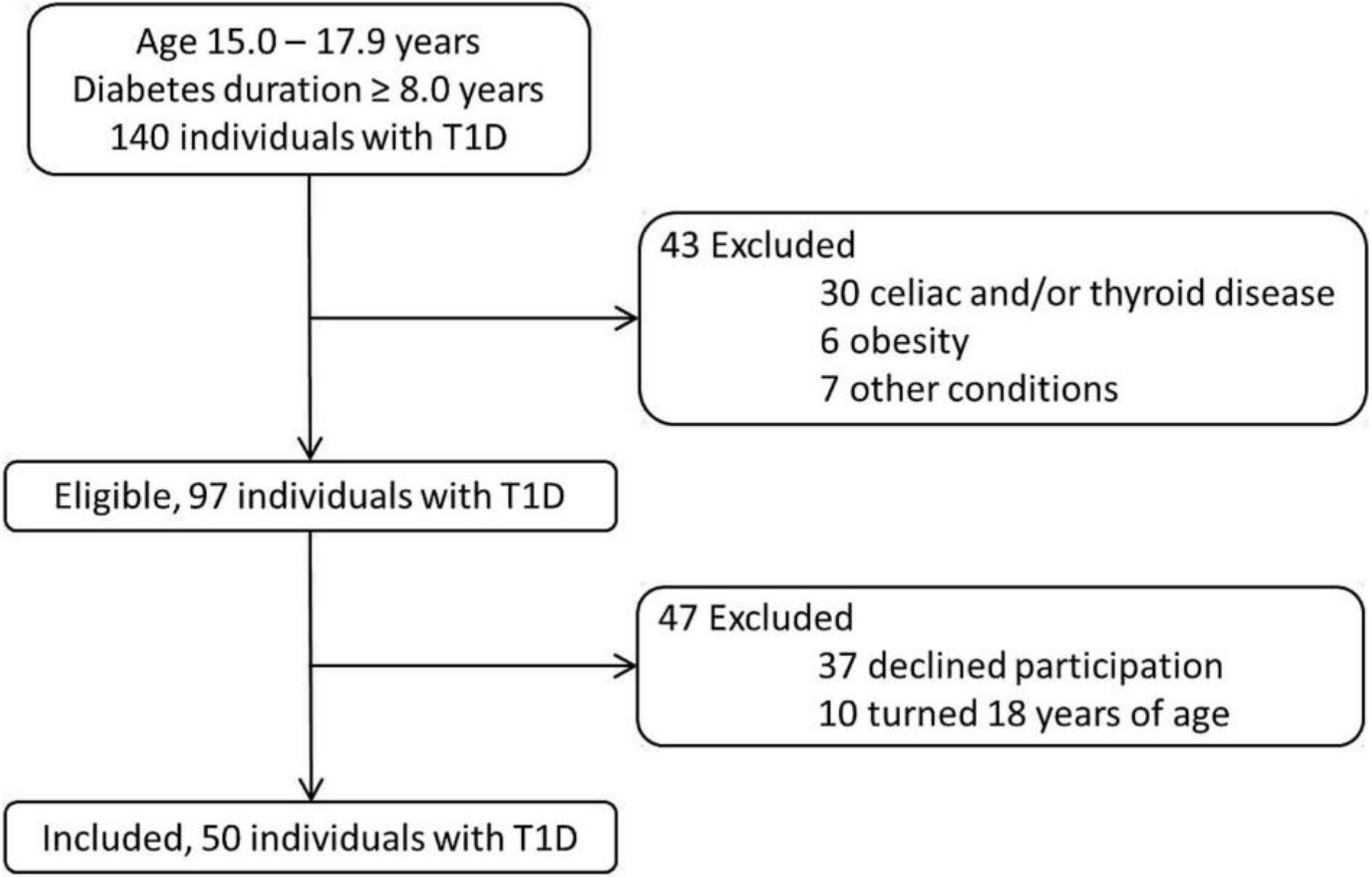
Age 15.0 – 17.9 years
Diabetes duration ≥ 8.0 years
140 individuals with T1D
43 Excluded
30 celiac and/or thyroid disease
6 obesity
7 other conditions
Eligible, 97 individuals with T1D
47 Excluded
37 declined participation
10 turned 18 years of age
Included, 50 individuals with T1D

Figure 1

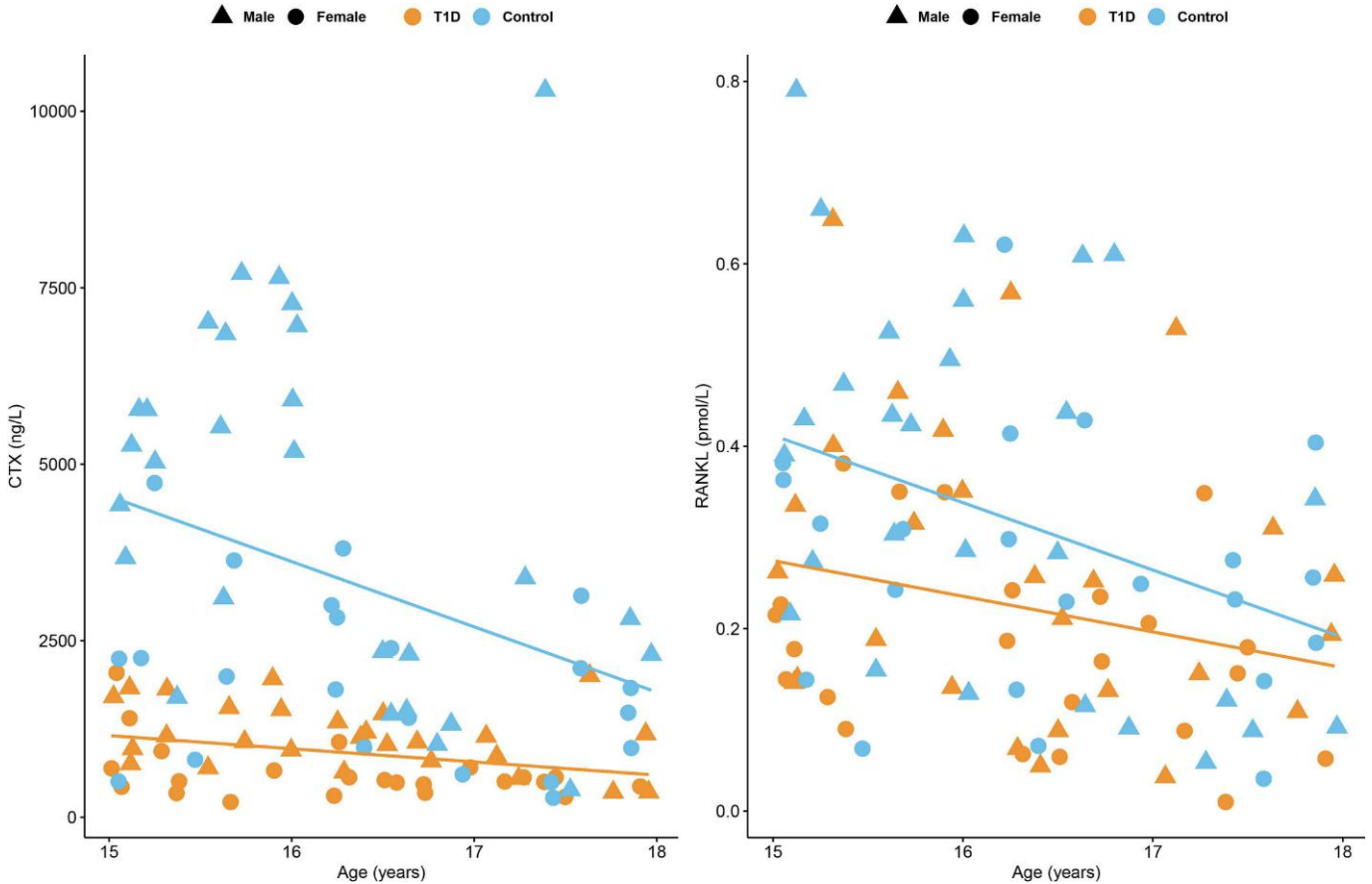
Male
Female
T1D
Control
CTX (ng/L)
10000
7500
5000
2500
0
15
16
17
18
Age (years)
Male
Female
T1D
Control
RANKL (pmol/L)
0.8
0.6
0.4
0.2
0.0
15
16
17
18
Age (years)

Figure 2