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Glycemic Control and Bone Mineral Density In Children and Adolescents with Type 1 Diabetes

Bone health in children with type 1 diabetes

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Abstract

Background/Aim: Fracture risk is increased in patients with type 1 diabetes. We aimed to evaluate bone mineral density (BMD) and to identify risk factors associated to lower BMD in Danish children and adolescents with type 1 diabetes.

Methods: In this cross-sectional study, BMD and BMD Z-score were determined by dual-energy X-ray absorptiometry (DXA) from a cohort of otherwise healthy children and adolescents with type 1 diabetes. Puberty Tanner stage, HbA1c, disease duration and age at diabetes onset were investigated for associations to DXA results.

Results: We included 85 patients, 39 girls, 46 boys, with a median (range) age of 13.2 (6-17) years; disease duration 4.2 (0.4-15.9) years; HbA1c of the last year 61.8 (41-106) mmol/mol. Our patients were taller and heavier than the background population. When adjusted for increased height SD and BMI SD, no overall difference in BMD Z-score was found. When stratified by sex, boys had significantly increased adjusted mean BMD Z-score, 0.38 (95%CI 0.13;0.62), girls; -0.27 (95%CI -0.53;0.00). For the whole cohort, a negative correlation between mean latest year HbA1c and BMD Z-score was found, adjusted ß -0.019 (95%CI -0.034;-0.004, p=0.01). Poor glycemic control (HbA1c >58 mmol/mol (7.5%)) within the latest year was likewise negatively correlated with BMD Z-score, adjusted ß -0.35 (95%CI -0.69;-0.014, p=0.04).

Conclusion: Our study suggests that elevated blood glucose has a negative effect on the bones already before adulthood in patients with type 1 diabetes, although no signs of osteoporosis were identified by DXA.

Keywords

Diabetes Mellitus, Type 1

Bone density

Glycated hemoglobin A

Child

Adolescent

Abbreviations

BMC: Bone mineral content, BMD: Bone mineral density, DXA: Dual-energy X-ray Absorptiometry, HbA1c: Hemoglobin A_{1c}, TBLH: Total Body Less Head

Introduction

Type 1 diabetes is a severe, chronic autoimmune disease currently affecting more than 1 million children and adolescents worldwide [1]. The incidence of type 1 diabetes is increasing and the disease imposes a significant risk of long-term complications including nephropathy, neuropathy and vascular complications [2]. In addition, adults with type 1 diabetes have an up to six-fold increased risk of bone fractures [3]. In a large UK cohort, an increased fracture risk was observed in both men and women with type 1 diabetes already in childhood and adolescence (0-19years) [4]. This increase in fracture risk has also become a priority in recent ISPAD guidelines, advocating DXA assessment in adolescents with long disease durations[5].

The mechanisms of diabetic osteopenia and bone fragility in patients with type 1 diabetes are not fully understood. The pathogenesis is probably multifactorial and diabetes may indirectly affect the bone metabolism in a number of ways including hypogonadism, hypercalciuria, altered vitamin D metabolism, or through associations with other diseases that influences the bones, e.g. coeliac disease [3,6]. Adult patients with type 1 diabetes have low bone mineral density (BMD) in the spine and hip,

and are also showing signs of impaired bone formation with low levels of osteocalcin and increased bone resorption biomarkers [3]. Previous studies indicate, that children and adolescents with type 1 diabetes may also have impaired bone metabolism with reduced osteoblast and increased osteoclast signaling [7,8]. As bones are extensively modelled in childhood and adolescence, it may be hypothesized that bones are altered in children with type 1 diabetes. Additionally, as the incidence of type 1 diabetes peaks between the ages of 10–14 years, bone effects may occur before the attainment of peak bone mass, which potentially results in diabetic osteopenia [9,10]. However, there is little knowledge regarding the bones in children and adolescents with type 1 diabetes.

Using Dual-energy X-ray Absorptiometry (DXA), some studies have found a lower BMD or bone mineral content (BMC) in children and adolescents with type 1 diabetes [11-13], while others did not find such associations [14-16]. In conclusion, the sparse previous studies in this area show conflicting results. Therefore, further studies are needed to elucidate why children and adolescents with type 1 diabetes have an increased fracture risk. This is clinically relevant as fragility fractures and delayed bone healing in persons with type 1 diabetes leads to major skeletal complications, which reduces their quality of life [17].

The aim of this study was to contribute to the knowledge on bone mineral density in children and adolescents with type 1 diabetes by (I) investigating alterations in BMD Z-score estimated by DXA in otherwise healthy children and adolescents with type 1 diabetes, and (II) identifying factors associated with lower BMD Z-scores.

Methods

This cross-sectional study was conducted at H C Andersen Children's Hospital and Department of Endocrinology at Odense University Hospital, Denmark. The inclusion and examination of participants and execution of the scans were performed from April to September 2017. The Strengthening the Reporting of Observational Studies (STROBE) in Epidemiology guideline for reporting cross-sectional studies was followed[18].

Participants

The participants were invited by pediatricians in the outpatient clinic of our institution. Children aged 6 to 17 years diagnosed with type 1 diabetes were included. Children with any bone disease (e.g. infantile osteopetrosis, hemihyperplasia), presence of other chronic diseases that might affect bone

metabolism (e.g. celiac disease, thyroid disorder) and previous or current treatment with medication that could alter the bone metabolism (e.g. systemic glucocorticoids) were excluded [6,19,20]. In addition, patients unable to co-operate with the study protocol were also excluded.

Measurements

Interview and examination

Participants were interviewed on history of medication use (including specific questions about systemic glucocorticoids, asthma and epilepsy medication), daily intake of milk and use of supplements containing vitamin D, calcium or multi-vitamins. Body weight without clothes was measured using an electronic scale (Seca 861, Germany) with an accuracy of 0.1kg and height without shoes was measured in cm (Seca 213, Germany). Biological sex and ethnicity were registered. Puberty development was assessed a.m. Tanner by clinical examination [21]. Tanner stage was stratified into pre-puberty (stage 1), early puberty (stage 2-3) and late puberty (stage 4-5).

DXA

DXA is the golden standard of assessing bone mineral density and is a common diagnostic tool for osteoporosis in adults. DXA can also be used in children, but to avoid overestimation of bone mineral deficits, areal BMD scores are commonly compared to reference data for the same sex and age by calculating a BMD Z-score instead of a T-score.

A GE Lunar Prodigy DXA scanner (GE Medical Systems, Madison, WI) equipped with ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI) was used at the modality Total Body Less Head (TBLH), providing estimation of BMD, bone mineral content (BMC), bone area and BMD Z-score. BMD Z-score was calculated based on the American reference data used in clinical settings at our institution [22]. This reference data set was divided into ethnicity and we used Caucacian.

The machine was calibrated daily and quality ensured weekly as recommended by the manufacturer. The scans were performed without clothes and without any jewelry, censors or pumps. Scan subjects were placed as instructed by the manufacturer and all scans were performed by the same trained person.

HbA1c

Glycated hemoglobin (HbA1c) is measured approximately every third month as part of routine clinical review of the diabetes patient cohort at our institution. We used this as an estimate of how well the diabetes was controlled. HbA1c was measured by high-performance liquid chromatography as fraction of total hemoglobin A0 using either Tosoh G7 or G8 (Medinor, Broendby, Denmark) with reagents as recommended by the supplier.

Sample values and measuring dates are recorded in the Biochemical Department of our institution dating back to 2010. HbA1c was calculated as a mean HbA1c within the latest year and mean HbA1c of the entire disease period or latest seven years if diabetes duration was longer than data were available.

Medical record review

Medical records of all participants were reviewed to ensure that no medical conditions had been registered excluding the participant from the study. The records were also reviewed for diabetes complications, the date of diabetes debut (date of the first diabetes-related hospitalization) and current diabetes treatment. Height, weight and body mass index (BMI) were expressed as SD according to the current Danish growth reference [23].

Data collecting

All data were collected by the same researcher to exclude interobserver variance. Study data were collected and managed using REDCap electronic data capture tools within Odense Patient data Explorative Network (OPEN) hosted at University of Southern Denmark and Odense University Hospital [24].

Analyses

Statistical differences between boys and girls were analyzed using linear regression, Chi-squared test, Fischer's exact test, Students t-test or Rank-sum test as appropriate. Assumption of normal distribution was determined by the Shapiro-Wilk test. Statistical analyses were preformed using STATA version 15.0.

In contrast to BMD T-score, BMD Z-score is computed based on patient age, as BMD increases with age in childhood. BMD is, however, also increased with above-normal body size for age and overweight, which is not adjusted for in the BMD Z-score calculation. We therefore adjusted BMD Z-score for case-specific variation from the normal Danish growth references for height and BMI expressed as standard deviation (SD) in our model.

Patient factors of interest were sex (for BMD Z-score), Tanner stage, mean HbA1c within the latest year, mean HbA1c of entire disease duration (up to seven years), age at diabetes onset and duration of diabetes. These parameters were tested in univariable and multivariable regression analyses based on our clinically driven models. Significant variables ($p<0.05$) in the univariable analyses were included in the multivariable analyses.

We furthermore performed a sub-analysis dividing patients into well-controlled diabetes (HbA1c ≤58 mmol/mol or 7.5%) and poorly controlled diabetes (HbA1c >58 mmol/mol) based on mean HbA1c within the latest year.

All models were controlled with normal quantile plots and residual-versus-fitted plots. Statistical method and analysis were approved by a statistician affiliated to the Odense Patient data Explorative Network (OPEN).

Ethics

Oral and written consent from parents of children younger than 15 years and adolescents older than 15 were acquired before the physical examination and DXA were performed. DXA is associated with a maximum radiation dose of 0.015 mSv. According to the guidelines from the International Commission on Radiation Protection (ICRP) the risk from this radiation dose is considered insignificant [25]. The project was approved by the regional ethical committee of Southern Denmark (Project-ID: S-20160159) and by the data protection agency (Journal nr:18/44863).

Results

We included 85 children and adolescents with type 1 diabetes (Fig 1). Of these, 79 (93%) were treated with continuous subcutaneous infusion while 6 (7%) were treated with multiple daily injections. Diabetes complications were only registered in two participants who had minimal microalbuminuria. One participant was in treatment with ACE-inhibitor due to familiar essential hypertension, another was in treatment for familiar hypercholesterolemia.

The participants were mainly Caucasian (95%) and 46 (54%) were boys. Median age was 13.2 (range 6-17) years. Disease duration median was 4.2 years and mean HbA1c of the latest year was 61.8 mmol/mol (7.8%) (Table 1). According to the recent national growth reference, the children were significantly taller, heavier and had a higher BMI than the average Danish child of the same age. Only height of girls was not significantly increased. No differences in characteristics were found between boys and girls.

The overall mean BMD Z-score was significantly increased compared to the reference, driven by a pronounced increased Z-score in boys of 0.73 (95%CI 0.47; 0.99) (Table 2). Height and BMI of the children with type 1 diabetes were, however, larger than average children of same age compared to the Danish growth curves. This could explain the higher BMD Z-score. Therefore, we adjusted the BMD Z-score for case-specific deviation in height and BMI. After adjustment, the overall BMD Z-score was no longer increased, but when stratified by sex, boys still had a significantly higher than normal BMD Z-score, 0.38 (95%CI 0.13; 0.62), while girls had a trend towards lower than normal BMD Z-scores, -0.27 (95%CI -0.53; 0.00). The difference in adjusted BMD Z-score between boys and girls was statistically significant, p=0.006.

In univariable analyses of patient factors of interest, Table 3A, sex and average HbA1c in the latest year had an independent association with the adjusted BMD Z-score. HbA1c for the entire diabetes duration showed a trend towards the same pattern; β -0.020, p=0.062.

In multivariable regression, including significant variables from the univariant analysis (sex and latest year hba1c), a negative correlation between HbA1c of the latest year and adjusted BMD Z-score was

still present; β -0.019, p=0.012, Table 3B. In other words, an increase of 10 mmol/mol in latest year HbA1c associated with a decrease of 0.19 in BMD Z-score. When analyzed using clinical cut-offs, the adjusted BMD Z-score was lower in poorly controlled patients compared to well controlled patients (latest year HbA1c ≤58 *vs.* > 58 mmol/mol); β -0.35 (95%CI -0.69;-0.014), p=0.04.
No associations were found between BMD Z-score and age at diabetes debut or duration of diabetes in the regression models.

Around 64% of the invited patients with type 1 diabetes patients participated in the study. The main reasons for non-participation were comorbidities (17%) and lack of consent (16%), Figure 1.
The latter was by far explained by lack of time, because the examinations were performed in daytime school and working hours. We did not suspect non-participants of being different regarding glycemic control compared to participants.

Our study was sufficiently powered as a *post hoc* power calculation showed that we were able to detect a true difference in BMD Z-score of 0.024 per 1 mmol/mol change in HbA1c, given a 2-sided alfa=0.05, beta 0.80, n=85 and BMD Z-score SD =0.1.

Discussion

In this relatively large cross-sectional study, we found a normal BMD Z-score adjusted for height SD and BMI SD in children and adolescents with type 1 diabetes. The adjusted BMD Z-score showed a negative correlation to latest year HbA1c and poorly regulated diabetes, defined as HbA1c >58 (>7.5%).
When stratified by sex, an increased adjusted BMD Z-score was detected in boys, in contrast, girls a trend towards having a lower than normal adjusted BMD Z-score. We found no differences in patient characteristics and the observed difference in BMD Z-score between boys and girls with type 1 diabetes remained unexplained.

The observed intersexual difference in BMD Z-score has not previously been reported in children or adolescents with type 1 diabetes. Most studies conducted in this area have not presented data stratified by sex. Furthermore, DXA methodology differs considerably between studies, as most attention has been paid only to the femoral neck or lumbar spine. We adhered to The International Society for

Clinical Densitometry 2014 recommendation to use TBLH when performing bone assessment in children and adolescents [26]. In a small study, Mosso *et al*. found boys to have higher total body BMD Z-score (0.51) than girls (0.02) but this did not reach statistical significance [14]. Maggio *et al*. also found no difference in total body BMD in children and adolescents with type 1 diabetes compared to healthy controls, but the patients were not divided by sex [27].

A difference in BMD between sexes has been seen in adults with type 1 diabetes. Neumann *et al*. found premenopausal women to have a significantly lower total body BMD, hip BMD and femoral neck BMD compared to healthy individuals, while no difference was found in men [28]. The mean peak height velocity SD score have also been found to be more impaired in girls than in boys with type 1 diabetes [29]. Further studies are needed to clarify whether our sex-specific BMI Z-score difference represents a chance finding, or a methodological or biological cause.

We found that the latest year HbA1c and poor glycemic control had an independent inverse association with BMD Z-score. This negative correlation has been seen in some [11-13], but not all studies [16,30]. Most other studies only measured Hb1Ac at the time of scanning. Latest year HbA1c represents a better marker of a potential effect of long-term glycemic control. An association between high HbA1c and low bone formation and increased bone resorption have been reported in other human studies [16,27]. In hyperglycemic environments, advanced glycation end products (AGEs) are produced and associated with osteoblastic apoptosis, decreased osteoblast proliferation and increased osteoclast activation in cells studies [31,32]. Type 1 diabetes patients with increased HbA1c may also have reduced glucose entry into bone cells, as high HbA1c may reflect a predominance of subnormal circulating insulin levels. Although the mechanisms remain poorly understood, our findings support the hypothesis that poor glycemic control impairs healthy bone development already in children and adolescents with type 1 diabetes. However, no independent associations were found between disease duration, or age at diabetes onset, and BMD Z-score, consistent with previous studies [27,30]. It is not known, whether our type 1 diabetes patient group with lower BMD Z-scores (poor glycemic control, females) are at increased risk of osteopenia later in life. It is likewise unclear, whether improvements in glycemic control may improve the BMD Z-score on the individual level. Repeat DXA scan follow-up studies are warranted in research cohorts. If maintenance or improvements in BMD Z-score can be detected after improvements in glycemic control, this may become a novel motivating factor for children and adolescents with type 1 diabetes,

We detected no overall reduction in BMD Z-score assessed by DXA, indicating no increased fracture risk in this patient group [33]. However, a large UK study showed an increased fracture risk in Type 1 diabetes patients already < 20 years in both sexes. They also found HbA1c to be positively correlated with bone fragility, but this included children and adults not stratified by age groups [4]. Of note, the median HbA1c in the study was higher (68 mmol/mol) compared to our cohort (61.8 mmol/mol). Fractures were too infrequent compared to our cohort size to be monitored. Taken together, our DXA study did not explain increased fracture risk seen in children and adolescents with type 1 diabetes. Although the resistance to fracture depends on BMD, Vestergaard described a discrepancy between BMD and fracture risk in adults with type 1 diabetes [34], suggesting other risk factors in bones. Scan modalities evaluating bone architecture could be considered in future research in supplementation to DXA.

Our study has several strengths. Our cohort of children and adolescents with type 1 diabetes is one of the largest studied by DXA and had sufficient statistical power by *post hoc* analysis. The study cohort was considered representative of the pediatric type 1 diabetes population without coexisting relevant comorbidities. This allowed analysis of the associations between type 1 diabetes parameters and BMD (Z-score) without confounding comorbidities and medications. Further strengths included closely monitored HbA1c values within the latest seven years, using TBHL for the bone assessment, physician-determined (not self-reported) Tanner staging and the adjustment of BMD Z-score by body size.

Limitations included the observational nature of the study, missing detailed data on insulin treatment modalities and physical activity in the retrospect, and the inborn limitations on interpreting BDM Z-scores as an indicator of bone health. Measures of BMD by DXA may be subject to inaccuracy due to shape dependent variation in bone area. Previous studies have found the size of some bones of patients with diabetes to be decreased [35,36]. Regarding treatment, the vast majority of our children were treated with continuous subcutaneous infusion (pump therapy). Insulin dosing seems to be important for bone development as bone size was found to be positively influenced by insulin [35]. A review by Chiarelli *et al*. showed less impaired longitudinal bone growth of children with type 1 diabetes and the obtainment of normal and predicted final height with the improvement of diabetes regulation by modern insulin therapy [29]. The improvement in care by modern insulin therapy probably has a positive effect on bone mineralization, but in our and most other studies of BMD in children and adolescents, it is unclear how diabetes has been treated or the amount of daily insulin received.

The BMD Z-score was based upon normal material from Caucasian American children. There may be small unknown differences between Danish and American children, but this American cohort is also used in the clinical setting and has been assessed representable.

Conclusion

In our cohort of children and adolescents with type 1 diabetes, BMD Z-score was in total increased, but when adjusted for larger body size, no overall abnormality in BMD Z-score was seen. Boys had significantly higher than normal BMD Z-score both before and after adjustment for body size. This sex-specific finding was not explained by demographic or disease characteristics. Poor glycemic control within the latest year, measured by HbA1c, was correlated to decreased BMD Z-score.

Our study suggests that elevated blood glucose affects the bones negatively already before adulthood in patients with type 1 diabetes, although no signs of osteoporosis were identified by DXA. Thus, our study did not explain the increased fracture risk in children and adolescents with type 1 diabetes demonstrated by previous studies. Further studies are warranted to explain whether the increased fracture risk may be caused by structural changes in the bones.

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Data availability

The data generated and analyzed during the current study are available in Odense Patient Data Explorative Network (OPEN), project number 495, https://open.rsyd.dk/OpenProjects/openProject.jsp?openNo=495&lang=da

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Contribution statement

All authors have contributed to this article and the Vancouver rules were respected at all times.

Conflict of Interest

The authors have no conflict of interest to declare.

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Tables and figures

Table 1 – Demographic and clinical characteristics of the study population with type 1 diabetes

	All	Boys	Girls	Boys vs. girls p-value
Participants, *n* (%)	85 (100%)	46 (54%)	39 (46%)	
Etnicity, *n* (%)				0.6
Caucasian	81 (95%)	43 (93%)	38 (97%)	
Middle East	4 (5%)	3 (7%)	1 (3%)	
Age, *years*				
Median (range)	13.2 (6.5; 17.9)	12.7 (6.7; 17.7)	13.6 (6.5; 17.9)	0.3
Tanner stage, *n* (%)				0.3
Pre-puberty (stage 1)	19 (22%)	13 (28%)	6 (15%)	
Early puberty (stage2-3)	23 (27%)	12 (26%)	11 (28%)	
Late puberty (stage 4-5)	43 (51%)	21 (45%)	22 (56%)	
				0.2
Pre-puberty, *n*	19 (22%)	13 (28%)	6 (15%)	
Puberty, *n*	66 (78%)	33 (72%)	33 (85%)	
Height, *cm*				
Median (range)	162 (113; 200)	163 (125; 200)	162 (113;180)	0.2
Z-score[a]	0.32 (0.11; 0.54)	0.52 (0.24; 0.80)	0.08 (-0.24; 0.41)	0.08
Weight, *kg*				
Median (range)	54.4 (19.8; 121.7)	53.0 (21.9; 121.7)	56.0 (19.8; 80.6)	0.2
Z-score[a]	0.74 (0.53; 0.95)	0.75 (0.08; 1.03)	0.73 (0.39; 1.06)	0.9
BMI, *kg/m²*				
Median (range)	20.0 (13.6; 38.0)	19.9 (13.6; 38.0)	20.3 (15.5; 27.6)	0.7

Z-score[a]	0.71 (0.48; 0.95)	0.61 (0.26; 0.95)	0.85 (0.54; 1.16)	0.3
Use of calcium supplements, *n* (%)	3 (3%)	2 (4%)	1 (3%)	1.0
Use of vitamin D supplements, *n* (%)	6 (7%)	4 (9%)	2 (5%)	0.4
Use of multivitamin supplements, *n* (%)	16 (19%)	7 (15%)	9 (23%)	0.4
Milk consumption *n* (%)				
Yes	80 (94%)	43 (94%)	37 (95%)	1.0
>2 glasses/day	33 (39%)	12 (26%)	21 (54%)	0.1
Use of asthma medication, *n* (%)	6 (7%)	2 (4%)	4 (10%)	0.4
Diabetes duration, *years*				
Median (range)	4.2 (0.4; 15.9)	4.1 (0.4; 15.9)	4.3 (0.4; 14.1)	0.7
Diabetes debut age *year*				
Mean (SD)	7.9 (3.5)	7.8 (3.7)	8.1 (3.3)	0.7
HbA1c, mean of disease duration				0.3
Median (range) *mmol/mol*	62.0 (47.3; 88.6)	60.9 (47.3; 88.6)	62.6 (48.6; 86.9)	
Median (range) **%**	7.8 (6.5; 10.3)	7.7 (6.5; 10.3)	7.8 (6.6; 10.1)	
HbA1c mean of latest year,				0.9
Median (range) ***mmol/mol***	61.8 (41; 106)	61.9 (41; 100.8)	61.0 (48.6; 106)	
Median (range) **%**	7.8 (5.9; 11.8)	7.8 (5.9; 11.4)	7.7 (6.6; 11.8)	

Chi2, Fischer's exact, students T-test and rank sum test as appropriate

[a]*SD from mean height/weight/BMI on growth reference (95%CI)*

Table 2 – DXA results in 85 children and adolescents with type 1 diabetes

DXA results	**All**	**Boys**	**Girls**	**Boys vs. girls p-value**
BMD Z-score				
Mean (95%CI)	0.42 (0.22; 0.62)	0.73 (0.47; 0.99)	0.06 (-0.21; 0.33)	**<0.001**
Adjusted BMD Z-score[a]	0.08 (-0.13; 0.30)	0.38 (0.13; 0.62)	-0.27 (-0.53; 0.00)	**0.006**

[a]*Regression with height SD and BMI SD adjusted to 0, to estimate the BMD Z-score if children had been normal size.*

Table 3 –Regression models for associations to DXA scan results

A) Univariable regression		**Coef. (95%CI)**	**p-value**
Adjusted BMD Z-score[a]			
	Sex *(ref.: girls)*	**0.642 (0.315;0.969)**	**<0.001**
	Tanner stage	-0.099 (-0.323;0.126)	0.4
	Age at diabetes debut, *years*	-0.009 (-0.059;0.041)	0.7
	Disease duration, *years*	0.006 (-0.041;0.053)	0.8
	HbA1c mean in duration (up to seven years), *mmol/mol*	-0.020 (-0.042;0.001)	0.062
	HbA1c latest year, *mmol/mol*	**-0.020 (-0.036;-0.004)**	**0.017**
B) Multivariable regression		**Coef. (95%CI)**	**p-value**
Adjusted BMD Z-score[b]			
	HbA1c latest year, *mmol/mol*	**-0.019 (-0.034;-0.004)**	**0.012**
	Sex	**0.634 (0.318;0.950)**	**<0.001**

[a]BMD Z-score adjusted for height SD, and BMI SD, R^2=0.39
[b]BMD Z-score adjusted for height SD and BMI SD, R^2=0.44
p=0.001 or p<0.001 for all covariable in adjustments

Figure 1 – Patient inclusion flow diagram