Bone turnover markers in children and adolescents with type 1 diabetes—A systematic review

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Bone turnover markers in children and adolescents with Type-1-Diabetes

— A systematic review

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Running title:

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Abstract:

**Background and objectives:** Type-1-Diabetes (T1D) is associated with impaired bone health and both osteocalcin and P1NP (markers of bone formation) and CTX (marker of bone resorption) are decreased in adult patients with T1D. We review the existing literature characterizing bone turnover markers in children and adolescents with T1D and by meta-analysis examine whether alterations in OCN, P1NP and CTX are evident and if potential changes correlate to the metabolic control (HbA1c).

**Subjects and methods:** Systematic searches at MEDLINE and EMBASE were conducted in January 2018 identifying all studies describing OCN, P1NP or CTX in children and adolescents with T1D. A total of 26 studies were included, representing data from more than 1000 patients with T1D. Pooled analyses of standard mean difference and summary effects analysis were performed when sufficient data were available.

**Results:** Pooled analysis revealed mean OCN to be significantly lower in children and adolescents with T1D compared to healthy controls (standard mean difference -1.87 95%CI: -2.83; -0.91) whereas both P1NP and CTX did not differ from the controls. Only data on OCN was sufficient to make pooled correlation analysis revealing a negative correlation between OCN and HbA1c (-0.31 95%CI: -0.45; -0.16).

**Conclusion:** OCN is decreased in children and adolescents with T1D. Whether CTX and P1NP are affected as well is unclear, due to very limited data available. New and large studies including OCN, P1NP and CTX (preferably as Z-scores adjusting for age-variability) is needed to further elucidate the status of bone turnover in children and adolescents with T1D.
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Key words:

Osteocalcin; Diabetes Mellitus, Type 1; Bone Remodeling; Child; Adolescent
Abbreviations:

T1D: Type 1 diabetes
T2D: Type 2 diabetes
BMD: Bone mineral Density
OCN: Osteocalcin
P1NP: Procollagen type 1 amino terminal propetide
CTX: C-terminal cross-linked telopeptide
FBG: Fasting Blood Glucose
SD: Standard Deviation
CSII: Continuous Subcutaneous Insulin Infusion
MDI: Multiple Daily Injections
MVD: Microvascular Disease
NTX: N-telopeptide of type 1 collagen
BMI: Body Mass Index
Introduction:

Bone health is affected by diabetes. Both Type-1-Diabetes (T1D) and Type-2-Diabetes (T2D) are associated with a higher risk of hip fractures, altered bone mineral density (BMD) and prolonged fracture healing time (1–3). In T1D, the BMD is significantly lower than in controls, whereas T2D patients have a higher BMD compared to controls (4,5). It has been suggested that these alterations of bone could be aligned with other long-term complications to diabetes, such as microvascular and neurological complications (6,7). Proposed mechanisms behind these alterations in BMD and fracture healing have been many and include altered calcium metabolism (8,9), decreased osteoblast maturation (10) and dissociation of the otherwise coupled formation and resorption of bone (11). Further it has been suggested that hyperglycemia, as seen T1D, impairs the anabolic effect of physical activity (mechanical stimulation) on bone potentially explaining the impaired bone seen in diabetes (12).

A recent review by Hygum et al. concluded that both markers of bone formation and bone resorption were decreased in patients with diabetes compared to healthy controls (5). Several markers were examined including the bone formation markers osteocalcin (OCN) and procollagen type-1 amino terminal propetide (P1NP) and the resorption marker C-terminal cross-linked telopeptide (CTX). The review included both T1D and T2D, but subgroup analysis confirmed that OCN and CTX were significantly lower in T1D compared to controls. The review did not make subgroup analysis based on age to examine the bone status in children and adolescents, separately. Only a limited number of studies have focused on the bone turnover markers in growing children and adolescents with T1D. However, several studies have concluded that even at young ages, BMD is lower compared to age and sex matched controls (13–18) and a recent review concluded that even in young and middle-aged adults with T1DM fracture risk remained increased (19).
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The incidence of T1D is highest in children and adolescents and more than 130,000 individuals under 20 years of age are expected to be diagnosed with T1D annually worldwide according to the International Diabetes Federation (20). A better understanding of the bone turnover in children and adolescents with T1D is of utmost importance due to the nature of skeletal development during growth. The bone turnover is continuously changing during growth and puberty and especially OCN is higher in children and adolescents compared to adults, with peak levels around puberty and the growth spurt (21,22). Hence, the consequences of impaired bone turnover during this period of growth could have potential life-long consequences. Further, if changes in bone turnover markers can be detected before alterations in BMD, they could have the potential to be markers of future impaired bone health.

Objectives

In this paper, we review the literature characterizing selected bone turnover markers in children and adolescents with T1D. We have chosen to focus on the classical markers of bone turnover (OCN, P1NP and CTX) for several reasons. First, these markers can be measured on automated platforms with high precision making them available in clinical practice (23). Secondly, these markers were among the most frequently reported amongst adults increasing the chances of sufficient data for meta-analysis (5). Finally, whereas P1NP and CTX have been recommended as prioritized markers of bone formation and resorption in clinical trials (by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine) (23), OCN was included due to the suspected close relationship to glucose metabolism as suggested by Lee et al. (24).

We focus on newly diagnosed patients during the remission phase as well as those with a longer duration of diabetes. The remission phase is characterized by initiation of insulin treatment and a typical temporary
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increase in endogenous insulin secretion, whereas the endogenous insulin is minimal in those with a longer disease duration.

The objective is to investigate whether the markers of bone turnover in a clinical setting can reveal alterations even at a very young age. We perform a meta-correlation analysis to evaluate the association between bone turnover markers and the glycemic control, represented by values of glycosylated hemoglobin (HbA1c).
Material and Methods:

The review was made using the PRISMA guidelines (25) and the full protocol has been registered in the International prospective register of systematic reviews (https://www.crd.york.ac.uk/prospero/)

(Prospero registration number: CRD42018089962).

Data sources and search strategy

A systematic literature search within the MEDLINE (at PubMed) and EMBASE databases were conducted on the 8th of January 2018 to identify all possibly relevant studies for the review. The search string was made with either MeSH terms or truncation according to the database. The final string combined the following search terms:

(("Diabetes Mellitus, Type 1") AND 
("Child") OR "Adolescent") AND 
((("Osteocalcin") OR "procollagen Type I N-terminal peptide") OR "collagen type I trimeric cross-linked peptide") OR “bone remodeling"

The full list of abstracts obtained from these searches was imported into the systematic review software COVIDENCE (https://www.covidence.org). The software facilitates the review process by removing all imported duplicates and structures the screening process of abstracts and full text articles.

Eligible criteria for study selection

Two of the authors (JOBM and JJ) screened all eligible abstracts against the inclusion criteria. Upon disagreement, the relevance of an abstract was decided by discussion.

Inclusion criteria were:
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- Studies should include separate results for children and/or adolescent spanning 0–20 years of age
- Subjects should be diagnosed with T1D
- Measurements of either OCN, P1NP or CTX should be presented in the article

Exclusion criteria included any other type of diabetes than T1D and any other condition or medication affecting the bone turnover i.e. steroids or recent/present fractures. Further, we excluded all animal studies and non-English-language articles.

Only full length, original articles were included. Abstracts from conferences were excluded in the full text screening if the results had not yet been published as full text articles. References were cross examined to include potentially overlooked publications fit for inclusion. If several publications were made by the same research group and the results were based on the same population, the most recent publication with the most complete set of results was included and the duplicates were excluded.

Data extraction and quality assessment

The final included articles were thoroughly examined, and the following data of interest were extracted if possible: mean age of participants, duration of diabetes, metabolic control (HbA1c), fasting blood glucose (fBG), measured bone turnover markers (OCN, CTX and P1NP), assays used to analyze bone turnover markers and whether the blood tests were done on fasting samples or not.

If necessary for analyzes and comparisons, extracted data were converted into the International units as following; OCN (µg/L), CTX (ng/L), P1NP (µg/L) and fBG (mmol/L). HbA1c will be reported in both mmol/mol and % in the text, however, in Table 1 the values are reported as they appear in the original manuscript.
If an included study was an intervention study the data from the baseline visit were included in our meta-analysis whilst the post-intervention data were excluded.

All included articles were evaluated using the Newcastle-Ottawa scale (NOS) being a tool developed to assess the quality of non-randomized studies in relations to design and content (26). For the cross-sectional studies a modified scale was used (27). Each study was rewarded between 0 and 10 points (9 for the cohorts) based on selection, comparability and outcome. Evaluation forms can be retrieved from:

http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

Statistical analysis

Mean values together with the 95% confidence intervals were retrieved from the articles. If data were published as subgroups, each subgroup was entered separately in the meta-analysis. Common mean differences were analyzed using the random effects model due to expected large degree of heterogeneity between studies. Heterogeneity between studies was estimated by I² analysis, estimating the percentage of variation between studies caused by heterogeneity (28). Test for publication bias was done visually using funnel plots. The meta-analyses were done using Review Manager Version 5.2.10.

Since bone turnover changes during childhood and adolescence in relation to growth, z-scores would have been a preferred presentation of bone turnover markers. However, only very few publications used z-scores, whereas most studies use age and sex matched controls to avoid confounding.

Summary effect analysis were calculated using Pearson correlation coefficients from studies presenting correlation analysis of HbA1c together with one or more of the bone turnover markers. Correlations coefficients were transformed to Fishes Z to obtain normal distribution before analysis. The meta-correlation
analyses were performed if four or more correlation coefficients, for a given bone turnover marker, were accessible. The meta-correlation analyses were done using RStudio Version 1.0.153.
Results

A total of 99 abstracts were identified in the databases and screening revealed 64 to be eligible for inclusion (Figure 1). 38 studies were then excluded after full text assessment, and the by far most common reason (N=24) being that the abstracts were from international conferences and had not yet made it to publication in scientific journals at the time of the literature search (8th of January 2018). Five articles were based on data from already included studies and contributed with no further information for this review. Other reasons for exclusion were articles in foreign languages or the included patients did not fulfill our inclusion criteria.

Unfortunately, one article could not be retrieved. Finally, three articles proved to be reviews, however, none of these focused on the same outcome and age group as in the current meta-analysis. Though our inclusion criteria stated that children and adolescents should be under the age of 20, one study by Leon et al. included adolescents up to the age of 21 years, but was kept in the analysis since the mean age was 11.2 (± 3.9) (29). Leaving out the study did not change the outcome or the significance of the pooled analysis (data are not shown).

In the end, 26 studies matched our criteria and were included; 20 cross-sectional studies (13,18,29–46) and 6 prospective cohort studies (17,47–51). One cohort by Saggese et al. was an intervention cohort study, but since we did not use post-intervention data, baseline data were used in the pooled meta-analysis (50).

Cross-sectional studies

As seen in Table 1, the number of participants varied greatly in the different cross-sectional studies; from 23 to 106 patients with T1D and from 11 to 420 controls. Mean age ranged from 8.0 to 15.5 years and the duration of T1D varied tremendously from 0 months to 18 years. Three studies did not include control groups (13,33,38). Most studies made it clear that the included participants and controls had no concurrent diseases, and only two articles did not state this (44,48). Seven studies did not state whether the blood tests...
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were drawn in a fasting state (17,32,37,39,40,45,49) and only 6 studies reported on blood glucose levels when tested, ranging from 9.3 to 16.52 mmol/L (29,34,35,40,42,46).

Osteocalcin was the most commonly analyzed of the bone turnover markers included in this review. 17 studies had measurements of OCN, whereas only eight studies reported on CTX and just four studies reported on P1NP. Seven studies included more than one of the bone turnover markers (33,34,36,37,41,42,47), and of these only two included all three markers (37,41). Though the specific assays differed, all bone turnover markers were analyzed by immunoassays. Four studies did not report HbA1c (31,32,36,48) and for the remaining the mean values ranged from 46 to 99 mmol/mol (6.4 to 11.2%).

DeSchepper et al. and Loureiro et al. used unusual units for their OCN measurements (ng/L and mg/dl respectively) (13,40). For both articles the corresponding authors were unsuccessfully contacted. Since the numeric values presented in the articles are similar to those from the other studies, and do not differ by a factor 100 or 1000 as the units would imply, it was perceived as mistakes. None of these studies entered the meta-analysis (for reasons specified later) and hence did not wrongly affect the pooled estimates. Khoshhal et al. presented CTX values for both T1D and controls approximately 50 times higher than those presented in other articles (37). No differences in units or obvious analytical methods could be identified to explain this difference. Excluding these results from the meta-analysis of CTX did not change the outcome (data not shown).

As seen in Table 1, NOS scores ranged between 4 and 9 points, with a mean of 7 points for both the cross-sectional studies and the cohorts. The most common cause of reduction in NOS scores was lack of information on recruitment and the sample representativeness of the study population.
The pooled analysis revealed that OCN levels were significantly lower amongst T1D children and adolescents (-1.87 (-2.83; -0.91)) whereas the difference in P1NP was insignificant (P=0.09) (Figure 2). The bone resorption marker CTX, was also without significant difference between T1D and controls (P=0.18).

Regarding all three markers of bone turnover, the heterogeneity between included studies was high and $I^2$ ranged between 95% and 98%.

Unfortunately, five studies could not be included in the pooled analysis. These studies were those without a control group (13,33) and those who presented data logarithmically transformed to obtain near normal distribution or those, that did not present results as mean and SD ie median and interquartile range (32,38,40). There is no reason to believe that the results from these omitted studies should change the overall outcome of the meta-analysis significantly. Three of the omitted studies had significantly lower values of OCN in T1D compared to controls (32,38,40) whereas one study just stated that OCN levels were within the normal range in T1D children and adolescents (13). The last study compared boys and girls with T1D (no control group), and observed that boys had higher levels of OCN and CTX without correcting for age or puberty (33).

One study reported on OCN in both the total and the undercarboxylated form and showed that both types were significantly lower in T1D compared to the control group. In the pooled analysis only data on total OCN were included (35).

Finally, because the time span of included articles is very wide and methods and assays for BTM analysis indisputably have evolved, the meta-analysis was repeated including only studies published after 2010. The
analysis did not change the outcome and still only OCN was significantly different in children and adolescents with T1D (-1.22; 95% CI: -3.65,-0.72) (data not shown).

Correlation analysis between OCN and HbA1c were reported in 13 of the included studies (29–33,35,38,40,43,47,51). Five of these studies did not publish data with a correlation coefficient (29,33,37,41,42) and one study only published a correlation coefficient for the undercarboxylated form of OCN (35). These studies could not be included in the pooled correlation analysis. The summary effects model for the remaining seven studies revealed a negative correlation between HbA1c and OCN -0.31 (95% CI: -0.45; -0.12), which was significant (P=0.0001) (Figure 3). Though not as pronounced as in the differences between means, the heterogeneity in the included correlation coefficients remained high with an I² score of 51.43%. Noteworthy, though two of the studies with no presented correlation coefficient also reported on negative correlation between OCN and HbA1c (41,42) the remaining three found no significant correlations (29,33,37). Only two studies reported on CTX and one on P1NP in correlation with HbA1c, and all of these showed negative correlations as well (41,42).

Cohort studies

To a higher extend than the cross-sectional studies, the included cohorts differed in design. Two studies followed recently diagnosed patients for one year with up to two follow-up visits in between. Pater et al. observed significantly lower OCN and LogCTX at onset but both markers normalized after 3 and 12 months (47). Reverse results were reported by Bonfanti et al. who observed CTX levels similar to the control group at onset of T1D, but significantly lower after 3, 6 and 12 months of disease (49).
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Gunczler et al. examined T1D patients with a diabetes duration of 4.3 (±2.9) years twice in one year. There were no difference in OCN in patients and controls at any time point and no change over time in any of the groups (17).

The last two studies investigated OCN at the time of onset. One study compared bone turnover markers before and after 15 days of insulin treatment (51) whereas the other described OCN in patients who presented with diabetic ketoacidosis at onset and again after normalized pH 10–15 days later (48). Both studies reported significantly lower levels of OCN in T1D at the time of T1D onset compared to the control groups, and both studies observed no difference between T1D and controls after 15 days of insulin treatment (48,51).
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Discussion

Our review shows that OCN is significantly lower in children and adolescents with T1D compared to healthy children and adolescents. Altered levels of P1NP and CTX was not observed. These findings suggest a shift, in the otherwise coupled bone turnover, towards impaired bone formation rather than increased bone resorption. Interestingly, at onset of T1D, levels of OCN were lower compared to controls but then normalized later in the remission phase.

Bone turnover markers and the metabolic control

Pooled correlation analysis showed a significant negative correlation between OCN and metabolic control in children and adolescents with T1D, indicating that an increase in HbA1c reduces bone formation. This may partially explain why decreased OCN is observed at the time of diagnosis, since this period naturally involves high levels of plasma glucose and hence increased HbA1c levels. When insulin therapy is initiated, and the blood sugar levels approaches normal levels, OCN levels normalize. However, the cause of these alterations remains to be determined.

Faienza et al. observed that patients treated with continuous subcutaneous insulin infusion (CSII) had significantly lower HbA1c compared to patients treated with multiple daily injections (MDI). Despite the lower HbA1c levels, the CSII-treated patients did not have different levels of OCN and CTX compared to the MDI-treated patients (34). Similar observations were made in other studies, but these studies could demonstrate a significant negative correlation between HbA1c and OCN, CTX and P1NP (36,41). The results from these studies imply that, even though levels of HbA1c is negatively correlated to levels of bone turnover markers, differences in HbA1c alone are not enough to alter the bone turnover, other factors must be involved. Interestingly, one study observed that even though T1D patients had significantly higher levels
of OCN compared to healthy controls, adjusting for age and body mass index (BMI) revealed a significant negative correlation between OCN and HbA1c (43).

Although it is generally agreed that HbA1c measurements should be used to assess metabolic control in patients with diabetes, HbA1c only reveals glycemic control within the preceding weeks to months. To obtain a more long-term idea of the glycemic control, some studies used averages of several measurements. One study used the mean of four HbA1c measurements from the past year whereas another used available measurements from the 2 preceding years (30,33). The study using the mean of four HbA1c measurements found a negative correlation between OCN and HbA1c as those mentioned earlier (33). The study using HbA1c measurements from the preceding two years found no significant correlation (30), suggesting that alterations in OCN might be more related to the current metabolic status than the past. Supporting this theory, studies made in children and adolescents at onset of T1D, observed that OCN levels went from significantly lower at onset, to be indifferent from the levels in the control groups, in just 10–15 days (48,51).

Altered bone turnover as a long-term complication

In healthy people, bone turnover is the ongoing process of coupled formation and resorption of bone. Impaired bone health, and even osteoporosis, develop if an uncoupling of the processes occur with an increase of resorption and/or decrease of bone formation (52). Our analyses indicated decreased bone formation in children and adolescents with T1D but no sign of altered bone resorption. These alterations may be the cause of the impaired bone health as seen in T1D (2,4). Impaired bone formation, for a given bone resorption, resulting in bone loss and decreased BMD was also suggested by Tsentidis et al. (42).

If altered bone turnover should result in long-term skeletal complications to T1D such as increased fracture risk and impaired fracture healing, disease duration would be expected to be an important factor. Just a few studies investigated the association to disease duration this(18,30,33,38,46). Two studies found that duration
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of T1D had a significant negative correlation with CTX (46) and OCN (30) whereas the rest found no correlation (18,33,38).

If both CTX and OCN decreases with an increasing disease duration, the theory of uncoupled bone formation and resorption in T1D might be wrong. However, the study, that found a negative correlation between disease duration and CTX, observed that CTX was significantly higher in patients with T1D for less than 1 year, compared to healthy controls, whereas, no difference was observed in patients with T1D for >1 and >5 years compared to controls (46). Again, if CTX decreased to the same level as the control group whereas OCN decreases to levels lower than controls, the bone turnover could still be perceived as uncoupled.

If changes in bone turnover markers precede changes in BMD in children and adolescents with T1D, we might consider screening to identify patients in risk of poor bone health as is done with other long-term complications to T1D (53). Only few studies have investigated bone health in relations to other long-term complications to T1D.

A large study, comprising 819 adolescents with T1D and a disease duration between 2–5 years, revealed that long-term complications were already present in up to 16% (54). Even if complications were present in the children and adolescents in the included studies, only Karaguzel et al. focused on this (36). Their subgroup analysis of participants with and without present microvascular disease (MVD) showed no difference in BMD regardless of the presence of MVD (36). They did, however, observe that both OCN and P1NP were significantly lower in T1D compared to controls. Unfortunately, they did not investigate OCN and P1NP levels in relation to presence of MVD.

In adults with T1D, Shanbhogue et al. evaluated bone microarchitecture using high-resolution peripheral quantitative computed tomography (HR-pQCT) (55). The study concluded that patients with long-lasting T1D without MVD had normal bone microarchitecture, while the presence of MVD was associated with
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impaired bone microarchitecture. Though there were no significant differences in OCN, P1NP and CTX between the patients with T1D with or without MVD, the bone turnover markers were all lower when comparing all patients with T1D to the control group (55). Another study in adults with T1D investigated bone health in relation to microalbuminuria. The study found that bone mass correlated negatively to microalbuminuria and the prevalence of microalbuminuria was increased in patients with BMD below the mean (56). The study further observed lower OCN in T1D compared to controls, but no difference in BMD (56).

The relationship between bone turnover markers and BMD in children and adolescents with T1D was investigated in three articles (17,33,37). Khoshhal et al. found a significant positive correlation between OCN and BMD, whereas the other two studies observed no correlation at all (37). Noteworthy, the participants in the study by Khoshhal et al. were all prepubertal with a mean age of 7.99 years, whereas the other two studies had mean ages of 12.1 (17) and 15.5 (33) years which could be a reason for the conflicting results.

As the data is limited, the results on the relationship between markers of bone turnover and BMD and bone architecture remains unclear. A possible explanation of the conflicting results might lay in the fact that markers of bone turnover changes rapidly ie in response to food, BMD and architecture changes slowly and are more likely to be a product of previous changes in turnover more so than the snapshot of bone turnover available from one measurement.

Bone turnover, age and puberty
Because of the skeletal development during growth in childhood and adolescence, age is an important factor when investigating bone turnover markers (21,22). One study showed higher levels of OCN and CTX in boys with T1D compared to girls with T1D (33). Though boys and girls were not different in age, the study reported that girls had entered puberty at an earlier age than boys (33). Because bone turnover changes during puberty and peaks during the growth spurt, girls entering puberty (and the growth spurt) earlier than boys, may very well be the cause of the observed differences. The theory is supported by the same study in which it is further observed that Tanner stage negatively correlates to both OCN and CTX (33).

One study divided patients with T1D and controls into groups based on Tanner stages. The study showed that OCN was lower in T1D in Tanner stage I through III and CTX and P1NP were lower in T1D in Tanner stage I compared to controls (41). Interestingly, BMD was not different from controls before the very end of puberty (Tanner IV–V) (41). Again, this suggests that alterations in bone turnover markers could be a precursor for changes in BMD.

Strengths and limitations

This review has some obvious limitations. First, the number of studies investigating bone turnover markers in T1D children and adolescents was very limited and most of these were cross-sectional. Especially studies investigating CTX and P1NP were missing, and only seven studies reported data on more than one of the markers OCN, CTX and P1NP making analysis of their interactions difficult. Secondly, the included studies were quite heterogeneous. The pooled analysis revealed unambiguous heterogeneity with $I^2$ test ranging from 95–98% (28). This could naturally be due to the large time span in the publication year of the studies (from 1986 to 2017), however, sub-analysis excluding data published data prior to 2010 (to limit variation in techniques and methods) did confirmed the overall finding. Several other factors may contribute to the large
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heterogeneity including differences in age and disease duration in the included patients. Further, pre-analytical conditions largely affect the markers of bone turnover, i.e. the fact that several articles abstain from reporting on the fasting state of their participants, could pose as a confounder when comparing the results (57). Several other factors such as geography, season of measurement, recent fractures and physical activity could also influence the comparisons of the results and should ideally be taken into account (57).

We know bone turnover changes dramatically during puberty and growth spurt (58). Heterogeneity could not be solved by further subdivision of data, owing to the limited data available at present, so we had to accept the use of a random effects model. Some “outlayer” observations were found in the presented data as two articles used unusual units in their OCN results (ng/L and mg/dl) which was not clear from looking at the actual numbers and another article presented inexplicable high values of CTX for both T1D and controls (13,37,40). None of these results affected the results of meta-analysis but stresses the need for larger investigations.

Funnel plots were made to assess the risk of publication bias. The funnel plots were quite asymmetrical for all three markers (OCN, CTX and P1NP) but especially for CTX and P1NP where the number of publications were very limited. This result was not surprising. A clear example of publication bias was identified in our pooled correlation analysis. Three out of five studies not publishing their correlation coefficient identified no correlation, but since there were no estimates, they could not be included in the pooled correlation analysis (29,33,38). Since no consensus exits on whether the bone turnover markers in T1D should be lower, higher or equal to controls, there should not be a predisposition to publish any specific results.

In this review, we focused solely on OCN, CTX and P1NP though other factors are involved in bone health and believed to play a part in the altered bone status of T1D. Some of these factors are analyzed in the
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included studies, together with OCN, CTX and P1NP, and includes vitamin D and PTH. These well-known influencers of calcium metabolism and bone health showed inconsistent results with levels equal to controls in most studies for both vitamin D (13,18,37,41,42,44,50) and PTH (13,18,29,34). However, low (36,42,50) and high PTH levels (44) were reported along with high (34,36) and low (35) vitamin D levels. Other markers examined in the included studies were physical activity and calcium intake (41,42), insulin-like growth factor (32,42) and the regulatory hormones from fat tissue, leptin/adiponectin (43,46). Further, new biological markers of bone turnover can be related to the changes observed in T1D, including sclerostin, osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-ligand (RANKL) (40,42–44). So even though the number of studies included is relatively small, they tend to focus on a wide pallet of players in bone health. Larger studies examining several factors simultaneously are warranted, ideally prospective cohort studies where patients are followed from onset of diabetes and onwards.

Conclusion

In conclusion, we find that OCN is decreased in children and adolescents with T1D. OCN is decreased already at the time of onset of T1D, normalizes after initiation of insulin treatment, and then after having had T1D for a while, OCN again decreases compared to controls. CTX and P1NP do not differ significantly from healthy children and adolescents, but data on these bone turnover markers are sparse. A wide range of factors seem to influence the markers of bone turnover, including age, gender, puberty, growth, BMI, metabolic control and disease duration. Larger studies are needed to elucidate the relationship between T1D and bone turnover markers in growing children and adolescents further. Studies with a more detailed focus on all markers and their relationship to each other, metabolic control and BMD from onset and further on are needed. Further, prospective studies looking at the causal relationship between metabolic control, bone...
turnover markers and BMD would add important new information to the current knowledge on T1D. Finally, the use of z-scores by age and gender for the bone turnover markers in children and adolescents seems mandatory to obtain valid comparisons.

Conflicts of interest:

No conflicts of interest.

References:


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Legends:

FIGURE 1: Flowchart of the article screening procedure reducing the number of articles from the potential 227 articles to the final 26 articles.

TABLE 1: Overview of included studies divided into cross-sectional and cohort studies and further subdivided according to analyzed bone turnover marker. Studies are listed with name of the first author and the year of publication. N= number of participants and controls (if any) and N Girls = number of girls in the study. Method = method of analyzing bone turnover marker. Units = units that the article has presented their results in. NOS = Newcastle-Ottawa Scale. P = significance level. P<0.05 is considered significant. N/A = Data not available. NS = Non-significant. CTX values presented by Pater et al were Logarithmically transformed. Age, duration of T1D in years, HbA1c, CTX, P1NP and OCN are all presented as mean ±SD unless otherwise stated. For comparison, the non-logarithmically results are chosen if available. IQR= interquartile range. SEM = standard error of mean. * is used when the P-value is only calculated from the logarithmically changed data.

FIGURE 2: Pooled analysis of OCN, CTX and P1NP in children and adolescents with T1D compared to healthy age matched controls. Studies with subgroups are marked with a number after the author name and year of publication. The following subgroups are entered: (1) Boys; (2) Girls; (3) Boys; (4) Girls; (5) HbA1c < 53 mmol/mol (< 7%); (6) HbA1c 53-75 mmol/mol (7-9%); (7) HbA1c >75 mmol/mol (9%); (8) Boys; (9) girls; (10) Disease duration < 1 year; (11) Disease duration 1-5 years; (12) Disease duration > 5 years.
FIGURE 3: Results from the pooled correlation analysis using the available Pearson correlation coefficients and the sample sizes. Using a random effect model, we got a summary effect size of -0.31 with a P-value of 0.0001.
227 references imported for screening
128 duplicates removed
99 studies screened against title and abstract
35 studies excluded
64 studies assessed for full-text eligibility
38 studies excluded

24 Conference abstracts only
5 Populations were used in other included studies
4 Wrong patient groups
3 Review Articles
1 Could not be obtained
1 Article not in English

26 studies included
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<th>Author, Year</th>
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<td>Guarneri, 1993</td>
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</tr>
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<td>Pater, 2010</td>
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<td>Wedrychowicz, 2014</td>
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**Correlation Coefficient**

pedi_12853_figure_3.eps
Table 1:

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**Bone turnover in pediatric diabetes**

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<th>Ref</th>
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<th>N Girls</th>
<th>Age in years</th>
<th>Duration in years</th>
<th>HbA1c in %</th>
<th>Bone turnover marker unit</th>
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<td>19.2 ±8.5</td>
<td>18.6 ±8.3</td>
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- **Mean ±SD (95% CI or Median (IQR))**
- **Mean ±SEM (Range) or Mean ±SEM or Range**
- **Median (IQR)**
- **Mean ±SD; Median (Range or Median (IQR))**
- **Mean ±SD; Median (Range) or Mean ±SD; Median (Range or Median (IQR))**
- **N/A**
- **NS**
- **P < 0.001**
- **P < 0.01**
- **P < 0.05**
## Bone turnover in pediatric diabetes

<table>
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<tr>
<th>Study</th>
<th>Ref</th>
<th>N</th>
<th>N Girls</th>
<th>Age in years</th>
<th>Duration in years</th>
<th>HbA1c in %</th>
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<tr>
<td>Khoshhal 2015</td>
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<td>36 T1D + 39 Control</td>
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<td>356.5 ±167.3</td>
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<td>11.4 ±1.4</td>
<td>Onset 3 months</td>
<td>11.3 ±2.4</td>
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<td>7.7 ±1.2</td>
<td>ng/mL</td>
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<td>14.5 ±3.2</td>
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<td>Onset 10–15 days</td>
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<td>N/A</td>
<td>N/A</td>
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<td>7.06 ±2.1</td>
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