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Different Health Behaviors and Clinical Factors Associated with Bone Mineral Density and Bone Turnover in Premenopausal Women with and without Type 1 Diabetes

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Abstract

Background—Women with type 1 diabetes (T1DM) have an elevated fracture risk. We therefore compared the associations of health behaviors and clinical factors with bone mineral density (BMD) and bone remodeling between premenopausal women with and without T1DM to inform potential interventions.

Methods—Participants included women with T1DM (n=89) from the Wisconsin Diabetes Registry Study and age- and race-matched controls without diabetes (n=76). Peripheral (heel, forearm) and central (hip, spine) BMD, markers of bone resorption and formation, bone cell signaling, glycemic control, and kidney function were assessed. Health behaviors and medical history were self-reported.

Results—In controls, but not in women with T1DM, older age was associated with lower bone resorption (p = 0.006) and formation (p=0.0007). Body mass index (BMI) was positively associated with heel and forearm BMD in both controls and T1DM women (all p<0.0001), but with hip and spine BMD only in controls (p = 0.005). Worse glycemic control during the previous 10 years, greater alcohol intake, history of smoking, and lack of physical activity were associated with poorer bone outcomes only in women with T1DM (all p = 0.002); whereas use of hormonal contraceptives was related to low bone formation in both women with and without T1DM (all p = 0.006). Diabetes duration, insulin dose, residual C-peptide, and kidney function were not associated with bone in T1DM.

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Conclusions—Age and BMI may not predict bone health in T1DM women. However modifiable behaviors such as optimizing glycemic control, limiting substance and hormonal contraceptive use, and increasing physical activity may improve bone health in T1DM women.

Keywords

type 1 diabetes; bone mineral density; bone turnover markers; glycemic control

Introduction

Individuals with type 1 diabetes (T1DM) are at higher risk of fracture than people without diabetes [1-3], and women with T1DM have the highest rate of fracture compared to men with T1DM and those without diabetes [4]. Studies examining bone health in premenopausal women with T1DM have concluded that bone mineral density (BMD) and bone formation (as determined by osteocalcin levels) are lower in women with T1DM than in women without diabetes [5-9].

Diabetes-specific factors such as glycemic control, duration of T1DM, age at T1DM onset, and the presence of complications may be related to bone health. While some studies have found no association of glycemic control with BMD [6,8], others have found an association of poor glycemic control with low BMD and altered bone turnover [5,9,10]. Duration of T1DM was negatively associated with BMD [11,12]. Earlier age at T1DM onset may also be associated with lower BMD [13]. With regard to complications, lower BMD in T1DM is associated with neuropathy, blindness, and nephropathy [7,14]. These studies are important for understanding diabetes-related causes of low BMD in this population, but few have examined the potential contributions of modifiable health behaviors to bone health in this high-risk population.

The associations of health behaviors and lifestyle factors such as diet, physical activity, and smoking with bone health are well documented and are central to many osteoporosis prevention programs [15,16]. Many studies of bone in T1DM have measured lifestyle and clinical factors such as diet, physical activity, smoking, alcohol intake, oral contraceptive use, family history of osteoporosis, and menstrual history as covariates [5,8,9], but their independent associations with bone health were not evaluated. The few studies examining health behaviors and BMD in adults with T1DM found BMD to be positively associated with physical activity [10] but negatively associated with milk intake [17], and to have null or negative associations with smoking [17,18]. The role of modifiable behaviors therefore needs to be elucidated in order to better educate premenopausal women with T1DM regarding their unique determinants of bone health to prevent fractures in this population.

The present analysis of premenopausal women with T1DM and matched controls was designed to address this research gap. This is the first study to our knowledge to systematically examine the associations of lifestyle, behavioral, and clinical factors with bone density and bone turnover in premenopausal women with T1DM, and to compare these associations with those in nondiabetic controls. Understanding how modifiable health behaviors contribute to bone health in premenopausal women with T1DM may provide opportunities for intervention and prevention of both bone loss and fractures.

Materials and Methods

Population and Sample

The Wisconsin Diabetes Registry Study (WDRS) is a population-based cohort of incident T1DM cases. Ascertainment and recruitment methods have been previously published [5,19]. Briefly, the WDRS enrolled participants (n=597) with T1DM diagnosed between May 1987 and April 1992, 30 years of age, and who lived in 28 contiguous counties in Wisconsin. T1DM was defined by the presence of polyuria and polydipsia, along with initiation of exogenous insulin therapy.

In 2005, 191 participants met the preliminary enrollment criteria for an ancillary study on bone health in T1DM: female, 18-50 years of age, premenopausal, continued participation in WDRS, and living in Wisconsin [5]. Through the end of recruitment, 89 eligible women with T1DM participated. The women were asked to identify a matched control (in priority order) as a full or half sister, female cousin, niece/aunt, or female friend without diabetes of the same race/ethnicity and within 5 years of age, up to 10 years if needed. Consequently, both groups were also matched on unmeasured genetic and lifestyle factors. Seventy-six controls participated. Study approval was obtained from the Institutional Review Boards at the University of Wisconsin-Madison and the University of Illinois at Chicago. Participants provided written informed consent at enrollment, at all subsequent study visits, and for the analysis of archived blood samples.

Data Collection and Variables

Blood Samples—Study visits were scheduled during days 20-24 of the menstrual cycle (luteal phase), and blood was collected via venipuncture without regard to fasting, or time of day or year. Immediately following the blood draw, a serum aliquot for osteocalcin determination was separated onsite and frozen in a portable -80°C freezer. Within a few hours of collection, the remaining blood was processed, centrifuged, and the serum or plasma separated. An aliquot of whole blood was refrigerated for determination of HbA1c, and an aliquot of serum was frozen at -80°C until being shipped to the lab for determination of N-terminal cross-linked telopeptides (NTx), as described below. Any remaining serum or plasma was archived for long-term storage at -80°C for additional bone turnover marker testing in the future, also described below.

Dependent Variables

Bone Turnover Markers: The following two bone turnover markers were measured in serum within four months of collection. Osteocalcin (bone formation marker) was analyzed by Fairview Diagnostic Labs (Minneapolis, MN) using a chemiluminescent immunoassay. NTx (bone resorption marker) was measured by Dynacare Labs (Milwaukee, WI) with an enzyme immunoassay. The intra- and inter-assay coefficients of variation (CVs) were 9% for osteocalcin and 14% for NTx.

Serum samples frozen at -80°C for five years were used to determine the following bone turnover markers. Bone-specific alkaline phosphatase (BAP; bone formation marker) and tartrate-resistant acid phosphatase 5b (TRAP5b; bone resorption marker) were measured by

Northwestern University's Comprehensive Center on Obesity: Metabolic Hormone Core Lab (Evanston, IL) by enzyme immunoassay with kits from Quidel Corporation (San Diego, CA). The intra- and inter-assay CVs were 7.6% for BAP and 3.0% for TRAP5b. Receptor activator of nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG; both markers of bone cell signaling) were measured by the Research Resource Core, University of Illinois at Chicago (Chicago, IL) by fluorescently labeled microsphere beads from Millipore (St. Charles, MO). The intra- and inter-assay CVs were 6.0% for RANKL and 6.5% for OPG. The manufacturer's insert for RANKL indicates that the kit can reliably detect values below 4.8 pg/ml using extrapolation. For this analysis, values ≥ 1.1 pg/ml were included as measured; for 13 women (5 T1DM, 8 controls) with values < 1.1 pg/ml, the value was set at 1.1 pg/ml [20]. To preserve the long-term stability of the bone turnover markers frozen to -80°C before testing, the number of freeze-thaw cycles was limited to two, fewer than all the assay manufacturers' recommendations.

One control did not provide consent to archive blood samples for future testing, and one woman with T1DM did not have enough archived serum for BAP, TRAP5b, RANKL, and OPG measurement. Two T1DM women had OPG values excluded because of extremely high levels ($> 6,700$ pg/ml). Because of intra-individual variability in bone turnover markers, data on the timing of the blood draw with regard to time of day and calendar month, time during the menstrual cycle, and time intervals since rising and since last food intake were collected for precision adjustment as needed in the analysis.

Bone Mineral Density (BMD): Bilateral heel (calcaneus) and forearm (distal radius/ulna) BMD were measured in all participants by Dual Energy X-Ray Absorptiometry (DXA) using the Peripheral Instantaneous X-ray Imager (PIXI, GE Lunar, Madison, WI). Means of bilateral heel and forearm measurements were analyzed. To validate peripheral measurements, hip and spine BMD were measured in a subgroup of women (26 T1DM, 23 controls) by DXA using a Prodigy densitometer (GE Healthcare Lunar, Madison, WI). The proximal femur of the left hip was measured and BMD of the total hip and femoral neck are presented. Lumbar spine BMD was determined using the antero-posterior projection and analyzed as the mean of L1-L4 vertebrae. Hip and spine BMD were significantly correlated with heel ($r=0.60 - 0.64$, all $p<0.0001$) and forearm ($r=0.43 - 0.52$, all $p = 0.002$) BMD irrespective of T1DM status. All measurements were conducted by one technologist. CVs of repeated measurements with repositioning were 3% at all sites.

Independent Variables

Health Behaviors and Clinical Factors: A stadiometer height rod and physician beam scale (Healthometer, Bridgeview, IL) were used to collect height and weight, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist-to-hip ratio was calculated by measuring waist and hip circumferences twice and averaging. A detailed questionnaire collected data on demographics; health behaviors (e.g., smoking history, daily alcohol and caffeine use, vitamin/supplement use, sleep habits, and weight change within the last year); reproductive history (e.g., age at menarche, average length of menstrual cycle, pregnancy history, and estrogen and/or progesterone hormonal contraceptive use); personal medical history (e.g., thyroid and celiac diseases, medications

for hypertension and kidney function, and family history of osteoporosis); and diabetes-specific factors for women with T1DM (e.g., diabetes duration, age at diagnosis, number of daily blood glucose checks, insulin dose, and use of an insulin pump). Calcium and vitamin D dietary intake were determined via a brief food frequency questionnaire [21]. Physical activity was measured with the Five-City Project physical activity assessment and quantified as daily total energy expenditure ($\text{kcalories} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Vigorous physical activity was defined as activity of at least six metabolic equivalents [22].

Glycemic Control and Insulin Resistance: Whole blood samples from all participants were analyzed for HbA1c, a measure of glucose levels during the previous 6-8 weeks, within seven days of collection by Fairview Diagnostic Labs (Minneapolis, MN). Automated high-performance liquid chromatography was used following the Diabetes Control and Complications Trial reference method [23]. The normal range for people without diabetes for this assay was 4.3-6.0%. The intra-assay CV was 0.6%; the inter-assay CV was 2.5-3.3%.

As a measure of current insulin resistance in women with T1DM, estimated glucose disposal rate (eGDR) was calculated as: $\text{eGDR} (\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = 24.31 - [12.22 \times \text{waist-to-hip ratio}] - [3.29 \times \text{hypertension status (yes=1; no=0)}] - [0.57 \times \text{HbA1c (\%)}]$ [24]. Lower values indicate greater insulin resistance.

Kidney Function: Cystatin-C was measured in all participants with archived serum, which had been frozen at -80°C for five years, using the Milliplex Human Kidney Toxicity Panel 2 kit (Millipore Corp., St. Charles, MO) with the Luminex system at the University of Illinois at Chicago (Chicago, IL). Again, one control did not provide consent to archive blood and Cystatin-C was not able to be determined in two T1DM participants due to insufficient archived serum. Extreme outliers (one T1DM, two controls) were excluded. The intra- and inter-assay CVs were 9%. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI Cystatin-C equation adjusted for age, sex, and race: $\text{eGFR} (\text{mL} \cdot \text{min}^{-1} \cdot 1.73\text{m}^2)^{-1} = 127.7 \times (\text{Cystatin-C (pg/ml)/1000})^{-1.17} \times \text{age}^{-0.13} \times 0.91$ [25].

Previously Collected Data Available from the WDRS for T1DM Women: Within 3-4 months after diagnosis, and again at four year follow-up, a subgroup of WDRS participants provided a 24-hour urine collection for C-peptide determination [26], as was the method used at that time [27]. Samples were tested within 20 weeks of collection as previously described, with creatinine measured to ascertain the completeness of specimen collection [28]. C-peptide at diagnosis and four year follow-up were respectively available on 51 and 57 women with T1DM in the current analysis.

At each previous WDRS follow-up visit, blood samples were obtained to determine total glycosylated hemoglobin (GHb). Total GHb measures the total amount of hemoglobin in the blood that is glycosylated, versus HbA1c that evaluates the glycosylation of only a specific subtype of hemoglobin. Since both measure glycemic control, they are highly correlated ($r=0.98$, unpublished data from WDRS, January 2002) [5]. Patients also submitted a blood sample from each visit with their primary care physician, or every four months if no visits were scheduled. Total GHb in the whole blood samples was measured within seven days of

collection by Isolab GlycAffin microcolumn affinity chromatography (Isolab, Akron, OH) as previously described [29].

These serial measurements of total GHb provided information on very long-term glucose control. The number of total GHb measurements per participant ranged from 1 to 18, with a mean of 7.8. The longitudinal glucose control for each participant was determined by calculating the mean and slope of their total GHb measurements from the 10 years prior to the ancillary bone study. One woman with T1DM was missing a 10-year mean and slope of GHb.

Statistics

Analyses were performed using SPSS Version 17.0 (IBM Corporation, Somers, NY) and SAS Version 9.2 (SAS Corporation, Cary, NC). Skewed dependent variables were log-transformed (e.g., NTx and RANKL) and independent variables with outliers were categorized (e.g., caffeine and alcohol intake quartiles) before analysis.

To account for the matching between women with and without T1DM (i.e., the non-independence of the two groups), comparisons of variables between the two groups used linear (PROC MIXED to compare means) and non-linear (PROC GLIMMIX to compare proportions) mixed regression models with a random intercept, specifying the matched pair number in the subject option. Next, stratifying the sample into women with T1DM and controls, ordinary linear regression was used to explore bivariate associations of BMD (heel, forearm, spine, femoral neck and total hip), bone resorption (NTx and TRAP5b), bone formation (osteocalcin and BAP), and cell signaling (RANKL and OPG) with demographics, health behaviors, and clinical factors. Multivariable linear regression models estimating BMD and the bone turnover markers for each group of women were built by entering all independent variables with $p \leq 0.15$ from the bivariate regression models, then using a backwards stepwise approach to retain only those variables with $p < 0.01$ (to limit type 1 error due to the multiple associations tested). Potential interactions between the retained independent variables were tested, but none were significant at $p < 0.01$. Any covariate with a significant association with BMD/bone turnover markers in women with T1DM but not controls, and vice versa, was tested for effect modification by diabetes status using linear mixed regression modeling accounting for the matching in the combined sample, and reported when significant.

Results

Participants

The characteristics of the sample are presented in Table 1. The sample was 98% non-Hispanic white with a mean age of 28.1 ± 7.5 years. Compared to controls, women with T1DM drank more caffeine but consumed less alcohol, had longer menstrual cycles and higher HbA1c, and demonstrated significantly lower peripheral BMD (forearm and heel) and osteocalcin levels.

Multivariable Models

Final multivariable models for BMD and bone turnover markers, stratified into controls and women with T1DM, are presented in Table 2.

Age and Adiposity—In controls, age (years) was negatively associated with NTx (nmol BCE/L (log), $\beta=-0.01$, $p=0.006$), TRAP5b (U/L, $\beta=-0.04$, $p=0.0003$), and BAP (U/L, $\beta=-0.37$, $p=0.0007$). Age was not associated with any bone outcome in women with T1DM. In controls, BMI was positively associated with BMD (g/cm^2) of the forearm ($\beta=0.004$, $p<0.0001$), heel ($\beta=0.007$, $p<0.0001$), spine ($\beta=0.016$, $p=0.0003$), and total hip ($\beta=0.012$, $p=0.005$), whereas in women with T1DM, BMI was positively associated only with peripheral BMD in the forearm ($\beta=0.003$, $p<0.0001$) and heel ($\beta=0.007$, $p<0.0001$), not with central BMD (hip and spine). Waist-to-hip ratio was not related to any bone outcome in either group.

Health Behaviors—In controls, average sleep (hours) was positively associated with OPG (pg/ml (log), $\beta=0.14$, $p=0.005$), but sleep was not associated with any bone outcome in women with T1DM. The interaction between sleep and the presence of T1DM on OPG was significant (interaction $p=0.0002$). In controls, caffeine intake (quartiles) was positively associated with spine BMD (g/cm^2 , $\beta=0.071$, $p=0.0002$). In women with T1DM, both alcohol intake (quartiles) and a history of smoking were negatively associated with NTx (nmol BCE/L (log), $\beta=-0.04$, $p=0.006$; $\beta=-0.08$, $p=0.007$, respectively). Vigorous physical activity ($\text{kcalories}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) was not associated with any outcome in controls but was positively associated with heel BMD in women with T1DM (g/cm^2 , $\beta=0.039$, $p<0.0001$). Calcium and vitamin D dietary intake and supplement use were not related to any bone outcome in either group.

Reproductive History—In controls, mean menstrual cycle length (22 to 39 days, 40 to 89 days, 90 days) was positively associated with BAP (U/L, $\beta=5.77$, $p=0.007$). For both controls and women with T1DM, estrogen+progesterone hormonal contraceptive use (never/former/current) was negatively associated with markers of bone formation, including osteocalcin (ng/ml, controls: $\beta=-1.08$, $p<0.0001$; T1DM: $\beta=-0.92$, $p<0.0001$) and BAP (U/L, controls: $\beta=-5.24$, $p<0.0001$; T1DM: $\beta=-2.84$, $p=0.006$). Additionally in controls, hormonal contraceptive use was positively associated with OPG (pg/ml (log), $\beta=0.20$, $p=0.005$). Pregnancy and breastfeeding history were not related to any bone outcome in either group.

Diabetes Factors in Women with T1DM—With regard to glycemic control, 10-year mean GHb (%) was negatively associated with BMD (g/cm^2) of the forearm ($\beta=-0.008$, $p=0.0007$) and heel ($\beta=-0.012$, $p=0.002$). The slope of the 10-year GHb measurements was negatively associated with BMD of the femoral neck ($\beta=-0.303$, $p=0.004$) and total hip ($\beta=-0.296$, $p=0.003$). There was a positive association between current HbA1c (%) and BAP (U/L, $\beta=1.41$, $p=0.004$). Age at diagnosis (years) was positively associated with OPG (pg/ml (log), $\beta=0.02$, $p=0.006$) and eGDR ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was positively associated with osteocalcin (ng/ml, $\beta=0.30$, $p=0.001$). Other diabetes factors including duration of T1DM, frequency of blood glucose checks, insulin dose, insulin pump use, C-peptide levels at

diagnosis and 4-year duration, and kidney function (Cystatin-C, eGFR, kidney function medications) were examined, but were not associated with any bone outcome.

Discussion

With the significantly elevated risk of fracture associated with T1DM [3], it is critical to elucidate factors associated with bone fragility in this group to target education and interventions. The current analysis demonstrated that some “traditional” risk factors for low BMD and altered bone turnover in nondiabetic populations were not associated with bone outcomes in women with T1DM. In the absence of diabetes, advancing age is associated with lower BMD and is an independent predictor of fractures [30]. In the control group of the current study, age was negatively associated with markers of bone resorption (NTx and TRAP-5b) and bone formation (BAP) but was not associated with any bone outcomes in women with T1DM. Slower skeletal remodeling would be expected with aging, but was not seen in women with T1DM. Higher BMI is also known to protect against bone loss [31]. In our sample, higher BMI was associated with greater peripheral BMD (heel and forearm) in women both with and without T1DM, whereas BMI was positively associated with central BMD (spine and total hip) only in controls. This suggests that higher BMI may not be of benefit to central BMD in women with T1DM. Whether and how T1DM actually alters the relationship of bone health with age and body mass is unknown; the lack of association of these traditional risk factors with bone outcomes in T1DM may rather be a function of other factors, such as glycemic control, having a stronger mechanistic role in bone health in T1DM.

Poor long-term glycemic control was consistently associated with lower BMD in young women with T1DM. Specifically, higher mean total GHb over 10 years prior to the bone assessments was associated with lower BMD at the heel and forearm, and an increasing trend in total GHb level over the 10 years was associated with lower femoral neck and total hip BMD. The regression coefficients quantifying the statistically significant decrement in BMD per one-percent increase in 10-year mean total GHb (Table 2) range from 13-17% of the standard deviation in heel and forearm BMD, respectively. This may be clinically relevant over the long-term as a one standard deviation lower peripheral BMD is associated with fracture risk ranging from 1.6-2.8 in nondiabetic postmenopausal women [32]. Our results are supported by basic science research which indicates that impaired biomechanical integrity of bone, including decreased bone mineralization [33], bone strength [34], and bone healing [33], results from hyperglycemia. High levels of advanced glycated end-products have also been found in bone collagen of diabetic animals, impairing osteoblast maturation [35], decreasing collagen strength, and increasing bone brittleness [36]. It is interesting to note that glycemic control was not associated with spine BMD, consistent with previous findings that the spine did not exhibit lower BMD in women with T1DM compared to controls [5,7], indicating the spine either may not be as affected by T1DM, or may take longer to exhibit changes.

The positive association of current HbA1c with BAP in women with T1DM is consistent with a recent meta-analysis of bone turnover markers in diabetes [37], and previous research specifically in premenopausal women with T1DM [9]. Indeed, cell studies demonstrate that

hyperglycemia directly alters the bone formation process [38,39], elevating BAP (expressed during the early phase of bone matrix maturation), and suppressing OC (expressed later as bone matrix is mineralized [40]). In addition, a lower ratio of OC/BAP is associated with vertebral fracture in type 2 diabetes [41]. In the present work, HbA1c was not associated with OC, but OC levels were significantly lower in the women with T1DM, and OC was positively associated with insulin sensitivity as measured by eGDR. These findings are consistent with novel research in mice demonstrating OC improves glucose tolerance [42]. Taken together, our results suggest that poor glycemic control, in addition to its association with micro- and macro-vascular complications, has negative impacts on bone formation and biomechanical integrity. It would follow that optimizing glycemic control may enhance mineralization of bone matrix and improve bone density, ultimately reducing fracture risk in women with T1DM. Bone fragility therefore needs to be recognized as an additional complication of T1DM that could potentially be prevented with improved glycemic control.

Along with glycemic control, the current study identified additional modifiable health behaviors associated with bone health in women with T1DM. Physical activity is known to preserve, even increase bone density [31], and vigorous physical activity was positively associated with heel BMD in women with T1DM. In the women with T1DM, greater alcohol intake and a history of smoking were related to slower bone resorption (NTx), but had no associations with bone formation. This is consistent with previous research demonstrating the negative impact of alcohol [43,44] and smoking [45-47] on bone repair and fracture risk. As bone remodeling begins with resorption, which subsequently triggers bone formation [48], slower bone resorption in T1DM women who drink alcohol and smoke may ultimately translate into altered bone remodeling and impaired bone repair. Taken together, these results may have significant clinical relevance. Health care providers would have another important reason to stress improved health behaviors in young women with T1DM. Greater physical activity and smoking and alcohol cessation by these women could help improve bone health and ultimately prevent fracture.

Former and current estrogen+progesterone contraceptive use was consistently associated with lower bone formation (OC and BAP) in women with and without T1DM. For controls, hormonal contraceptive use was positively associated with OPG, similar to the trend found in previous research [49]. Hormonal contraceptives may therefore be slowing the initiation of bone turnover, and subsequent bone formation, as OPG blocks the development and activation of osteoclasts [50]. However, the associations of hormonal contraceptive use with altered bone remodeling did not extend to bone density in either group of women. These results are consistent with previous research in premenopausal women demonstrating the relationship of oral hormonal contraceptive use with lower bone formation [51,52] and BMD [53-55]. In light of our findings, health care providers may need to weigh the benefits of hormonal contraceptives with the potential negative impact on bone remodeling in women with T1DM.

One diabetes-specific factor associated with a measure of bone metabolism, but not modifiable, was age at T1DM diagnosis. We detected a positive association between age at diagnosis and OPG levels. Higher OPG levels signify slower bone turnover [50], so an older age at diagnosis may be associated with lower bone remodeling and BMD. In contrast,

previous research found that an earlier age at T1DM onset was associated with lower BMD [13,56]. Turning to a different body of literature, OPG is also a marker of cardiovascular disease [57] and is modulated by proinflammatory cytokines [58]. Therefore, OPG may represent a marker of inflammation in our current study of women with T1DM, and an older age at diagnosis may be related to greater inflammation. Consistent with this idea, research on microvascular complications in T1DM found that an older age at diagnosis was associated with accelerated development of microalbuminuria and retinopathy [59-61]. Taken together, OPG in T1DM women in the current analysis may reflect greater inflammation and not be specifically a marker of bone remodeling.

In control women, three associations with bone outcomes were interesting. First, more sleep per night was associated with higher OPG. As mechanical strain (e.g., weight bearing) increases OPG production [50], OPG again in this instance may not reflect bone remodeling. Prior research has reported that women with major depressive disorder have higher levels of OPG [62]. While a thorough health history was taken, a history of depression was not collected, and a subgroup of controls may have had undiagnosed depressive symptoms driving the desire to sleep and higher OPG. Second, BAP was higher in control women who had longer menstrual cycles. Women with polycystic ovary syndrome (PCOS) are more likely to present with longer menstrual cycles and androgen excess [63], and androgens have been shown to stimulate bone metabolism and BAP [64]. Again, the presence of PCOS and androgen excess in a subgroup of controls may explain the positive association between BAP and menstrual cycle length. Lastly, we detected a positive association between caffeine intake and spine BMD in controls. Previous literature has reported that caffeine intake is not associated with bone in young women [65,66]. However, tea consumption has been shown to be associated with greater bone mass [67], and the positive association we detected between caffeine and spine BMD may reflect the effect of tea and not caffeine.

The study's strengths include recruitment of the sample from a well-established population-based diabetes registry, with historical data on glycemic control. The T1DM sample was homogenous with respect to gender, race, menopausal status, and diabetes duration, and a matched control group consisting of family and friends minimized potential confounding. An in-depth questionnaire collected extensive data on health behaviors and medical history. Limitations include a 98% non-Hispanic white all female sample, so the results may not be generalizable to other races/ethnicities or to males. Hip and spine BMD were collected in only a subgroup of women with and without T1DM in order to validate heel and forearm BMD measurements in our sample. Research will be needed to confirm and expand upon our findings on health behaviors associated with hip and spine BMD using a larger cohort with T1DM. Data collection on health behaviors and medical history relied on self-report; actigraphy could be used as a more reliable and valid measure of physical activity and sleep in future studies to confirm our findings. In addition, recent studies have demonstrated lower bone quality in individuals with T1DM [68] and measures of bone quality with more advanced technology [69] should be included in future research.

To our knowledge, this is the first study to thoroughly examine the association of modifiable health and lifestyle behaviors with bone health in premenopausal women with T1DM. Optimizing glycemic control and vigorous physical activity may benefit, whereas alcohol

use, smoking, and hormonal contraceptives may compromise, bone health in women with T1DM. In addition, older age and lower BMI may not be risk factors for osteoporosis in women with T1DM. Premenopausal women with T1DM therefore have unique needs which require tailored bone health education, and these identified modifiable behaviors offer several opportunities for nurses, physicians, dietitians, personal trainers, and pharmacists to educate this population and improve bone health. Figure 1 presents a summary of the key findings of this study, which may help educate women with T1DM on potential benefits and risks to bone health. Additional studies are needed to confirm our findings, and interventions targeting these modifiable factors should then be developed and studied for their effectiveness.

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Figure 1.
Summary of Factors Associated with Bone Health in Women with T1DM

Table 1

Characteristics of Premenopausal Women with and without T1DM

	T1DM (n=89)		Controls (n=76)	
Age (years)	27.9	(6.8)	28.4	(8.2)
Body mass index (kg/m ²)	28.1	(6.0)	27.1	(6.1)
Waist-to-hip ratio	0.78	(0.06)	0.77	(0.05)
Average sleep per night (hours)	7.1	(1.2)	7.1	(1.0)
Calcium intake (quartiles mgs/day), n (%)				
81 to 477	21	(24)	20	(26)
478 to 939	20	(22)	22	(29)
940 to 1367	23	(26)	18	(24)
1368 to 3441	25	(28)	16	(21)
Vitamin D intake (quartiles IUs/day), n (%)				
0 to 68	25	(28)	16	(21)
69 to 170	20	(22)	22	(29)
171 to 417	22	(25)	19	(25)
418 to 1358	22	(25)	19	(25)
Caffeine intake (quartiles mgs/day), n (%)				
0 to 33	17	(19) *	24	(32)
34 to 99	18	(20)	24	(32)
100 to 168	28	(32)	13	(17)
169 to 710	26	(29)	15	(20)
Alcohol use (quartiles g/day), n (%)				
0 to 1.1	31	(35) †	14	(18)
1.2 to 3.1	21	(24)	17	(22)
3.2 to 8.3	21	(24)	20	(26)
8.4 to 79.2	16	(18)	25	(33)
Taking calcium supplement, n (%)	38	(43)	37	(49)
Taking vitamin D supplement, n (%)	30	(34)	28	(37)
History of smoking, n (%)	51	(57)	43	(57)
Vigorous physical activity (kcalories•kg ⁻¹ •day ⁻¹), n (%)				
None	40	(45)	29	(38)
0.1 to 2.6	26	(29)	29	(38)
2.7	23	(26)	18	(24)
Mean menstrual cycle length (days), n (%)				
22 to 39	66	(74) †	71	(93)
40 to 89	12	(14)	2	(3)
90	11	(12)	3	(4)
Estrogen+progesterone contraceptive use, n (%)				
Never	24	(27)	16	(21)
Former	33	(37)	30	(39)

	T1DM (n=89)		Controls (n=76)	
Current	32	(36)	30	(39)
eGFR (mL•min ⁻¹ •1.73m ² ⁻¹)	48.8	(27.3)	46.8	(37.6)
Current HbA1c (%)	8.1	(1.7) †	5.0	(0.3)
Current HbA1c (mmol/mol)	65.3	(18.4) †	31.1	(3.4)
10-year mean GHb (%)	10.8	(2.0)	—	
Diabetes duration (years)	16.0	(1.5)	—	
Age at diagnosis (years)	11.9	(6.7)	—	
Insulin dose (units/kg)	0.79	(0.45)	—	
eGDR (mg•kg ⁻¹ •min ⁻¹)	9.9	(1.7)	—	
Bone mineral density (g/cm ²)				
Forearm	0.440	(0.048) †	0.464	(0.052)
Heel	0.492	(0.092) †	0.533	(0.094)
L1-L4 Spine	1.203	(0.149)	1.254	(0.145)
Femoral Neck	1.032	(0.135)	1.070	(0.120)
Total Hip	1.027	(0.129)	1.064	(0.117)
Bone turnover markers				
Osteocalcin (ng/ml)	3.7	(1.7) †	4.6	(1.7)
BAP (U/L)	24.9	(8.0)	23.3	(9.2)
NTx (nmol BCE/L; log)	1.0	(0.1)	1.0	(0.1)
TRAP-5b (U/L)	2.4	(1.0)	2.3	(0.9)
RANKL (pg/ml; log)	1.2	(0.6)	1.1	(0.6)
OPG (pg/ml; log)	7.1	(0.4)	7.1	(0.5)

BAP=bone-specific alkaline phosphatase; BCE=bone collagen equivalent; eGDR=estimated glucose disposal rate; eGFR=estimated glomerular filtration rate; GHb=glycosylated hemoglobin; NTx=N-terminal cross-linked telopeptides; OPG=osteoprotegerin; RANKL=receptor activator of nuclear factor κ B ligand; TRAP-5b=tartrate-resistant acid phosphatase 5b

Data presented are means (standard deviations) or n (%) T1DM: Spine, femoral neck, total hip BMD (n=26); TRAP-5b, BAP, RANKL, 10-year GHb (n=88); OPG, eGFR (n=86)

Controls: Spine, femoral neck, total hip BMD (n=23); TRAP-5b, BAP, RANKL, OPG (n=75); eGFR (n=73)

Conversion to SI units: HbA1c (mmol/mol) = (HbA1c (%) - 2.15) $\times$ 10.93; Osteocalcin (nmol/L) = Osteocalcin (ng/ml) $\times$ 0.17; OPG (pmol/L) = OPG (pg/ml) $\times$ 0.05

*
p < 0.05

†
p < 0.01

Table 2

Multivariable Models of Bone Mineral Density and Bone Turnover Markers in Premenopausal Women with (n=89) and without (n=76) T1DM

	Bone Mineral Density (g/cm ²)									
	Forearm		Heel		L1-L4 Spine		Femoral Neck		Total Hip	
	Controls	T1DM	Controls	T1DM	Controls	T1DM	Controls	T1DM	Controls	T1DM
Adjusted R ²	0.20	0.31	0.21	0.43	0.61	—	—	0.27	0.28	0.28
Age and Adiposity										
Age (years)	—	—	—	—	—	—	—	—	—	—
Body mass index (kg/m ²)	0.004 [†]	0.003 [†]	0.007 [†]	0.007 [†]	0.016 [†]	—	—	—	0.012 [*]	—
Health Behaviors										
Average sleep per night (hours)	—	—	—	—	—	—	—	—	—	—
Quartiles of caffeine intake	—	—	—	—	0.071 [†]	—	—	—	—	—
Quartiles of alcohol use	—	—	—	—	—	—	—	—	—	—
History of smoking [‡]	—	—	—	—	—	—	—	—	—	—
Vigorous physical activity (kcalories•kg ⁻¹ •day ⁻¹) [§]	—	—	—	0.039 [†]	—	—	—	—	—	—
Reproductive History										
Mean menstrual cycle length //	—	—	—	—	—	—	—	—	—	—
Estrogen+progesterone contraceptive use ¶	—	—	—	—	—	—	—	—	—	—
Diabetes Factors										
Current HbA1c (%)	—	—	—	—	—	—	—	—	—	—
10-year mean GHb (%)	—0.008 [†]	—	—0.012 [*]	—	—	—	—	—	—	—
10-year GHb slope	—	—	—	—	—	—	—0.303 [*]	—	—0.296 [*]	—
Age at diagnosis (years)	—	—	—	—	—	—	—	—	—	—
eGDR (mg•kg ⁻¹ •min ⁻¹)	—	—	—	—	—	—	—	—	—	—
Bone Turnover Markers										
NTx (nmol BCE/L; log)	Controls	T1DM	Controls	T1DM	Controls	T1DM	BAP (U/L)	OPG (pg/ml; log)	Controls	T1DM
	—	—	—	—	—	—	—	—	—	—

Adjusted R ²	0.09	0.16	0.16	—	0.21	0.26	0.35	0.15	0.17	0.08
Age and Adiposity										
Age (years)	-0.01 [*]	—	-0.04 [†]	—	—	—	-0.37 [†]	—	—	—
Body mass index (kg/m ²)	—	—	—	—	—	—	—	—	—	—
Health Behaviors										
Average sleep per night (hours)	—	—	—	—	—	—	—	—	0.14 [*]	—
Quartiles of caffeine intake	—	—	—	—	—	—	—	—	—	—
Quartiles of alcohol use	—	-0.04 [*]	—	—	—	—	—	—	—	—
History of smoking [‡]	—	-0.08 [*]	—	—	—	—	—	—	—	—
Vigorous physical activity (kcalories•kg ⁻¹ •day ⁻¹) §	—	—	—	—	—	—	—	—	—	—
Reproductive History										
Mean menstrual cycle length //	—	—	—	—	—	—	5.77 [*]	—	—	—
Estrogen+progesterone contraceptive use ¶	—	—	—	—	-1.08 [†]	-0.92 [†]	-5.24 [†]	-2.84 [*]	0.20 [*]	—
Diabetes Factors										
Current HbA1c (%)	—	—	—	—	—	—	—	1.41 [*]	—	—
10-year mean GHb (%)	—	—	—	—	—	—	—	—	—	—
10-year GHb slope	—	—	—	—	—	—	—	—	—	—
Age at diagnosis (years)	—	—	—	—	—	—	—	—	—	0.02 [*]
eGDR (mg•kg ⁻¹ •min ⁻¹)	—	—	—	—	—	0.30 [*]	—	—	—	—

BAP=bone-specific alkaline phosphatase; BCE=bone collagen equivalent; eGDR=estimated glucose disposal rate; GHb=glycosylated hemoglobin; NTx=N-terminal cross-linked telopeptides; OPG=osteoprotegerin; TRAP-5b=tartrate-resistant acid phosphatase 5b Data presented are regression coefficients

T1DM: Spine, femoral neck, total hip BMD (n=26); TRAP-5b, BAP, 10-year GHb (n=88); OPG (n=86)

Controls: Spine, femoral neck, total hip BMD (n=23); TRAP-5b, BAP, OPG (n=75)

* p < 0.01

† p < 0.001

‡ 0 = no, 1 = yes

§ 0 = none, 1 = 0.1 to 2.6 kcalories•kg⁻¹•day⁻¹, 2 = 2.7 kcalories•kg⁻¹•day⁻¹

// 0 = 22 to 39 days, 1 = 40 to 89 days, 2 = 90 days

0 = never, 1 = former, 2 = current

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