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Evidence of disordered calcium metabolism in adolescent girls with type 1 diabetes: an observational study using a dual-stable calcium isotope technique

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Abstract

Type 1 diabetes (T1D) is associated with skeletal abnormalities including low bone density and increased fracture risk. The pathophysiology underlying T1D related skeletal fragility remains unknown. The objective of this study was to use a dual-stable calcium isotope method to investigate the effects of T1D on calcium absorption and estimated calcium retention in adolescent females. Twenty adolescent females with T1D were admitted for a 24-hour calcium absorption study using oral (⁴⁴Ca) and intravenous (⁴²Ca) stable isotopes for determination of percent gastrointestinal calcium absorption and estimated calcium retention. Five out of twenty participants were found to have negative estimated calcium retention. Participants with negative calcium retention had greater urinary calcium excretion [202 mg/d (IQR: 178–213)] compared to those with positive calcium retention [101.5 mg/d (IQR:82–122)], $p=0.01$, but similar calcium intake and percent calcium absorption. With the exception of one outlier, 24-hr urine calcium was significantly associated with hemoglobin A1c (Pearson's $r=0.55$, $p=0.02$). 50% of participants consumed less than the RDA for calcium; fractional calcium absorption was inversely correlated with calcium intake in participants not meeting the RDA (Spearman's $\rho -0.65$, $p=0.04$). In conclusion, one-quarter of adolescent girls with T1D were found to have negative estimated calcium retention at a time when bone mineral accrual should be ongoing. This appeared to be the result of excess urinary calcium excretion as opposed to diminished gastrointestinal calcium absorption. Insufficient calcium availability for bone deposition during adolescence could impair bone mineral accrual and contribute to skeletal fragility.

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Keywords

Type 1 diabetes; Calcium; Stable Isotopes; Hyperglycemia; Hypercalciuria; Bone mineral content

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease characterized by absolute insulin deficiency and hyperglycemia. The majority of patients living with T1D develop the disease during childhood [1] and are therefore exposed to the deleterious metabolic consequences of T1D for decades. In addition to the well-recognized effects of T1D on renal, cardiovascular, and nervous systems, it is now apparent that skeleton is also adversely affected. Multiple studies have shown that T1D is associated with an increased risk of fracture [2, 3].

Additional abnormal skeletal findings reported in T1D include deficits in bone mineral density (BMD) and bone microarchitecture, low bone formation markers, and impaired bone strength [4–7]. Osteoporotic fracture is associated with significant morbidity, mortality and health care cost [8]; yet there are no standard guidelines for the monitoring or treatment of bone health in this high-risk population. In part, this stems from a lack of understanding of the pathophysiology underlying impaired bone health in T1D.

Diminished calcium availability for bone mineral accrual during childhood and adolescence is one possible contributor to T1D related skeletal disease. Calcium is the primary mineral component of bone and contributes to bone strength [9]. Bone mineral content (BMC) accrual and bone calcium accretion peak during adolescence, with approximately one-third of BMC accrual occurring during the years surrounding the pubertal growth spurt [10]. Since the incidence of T1D is highest during childhood [1]; most patients living with T1D will be exposed to any deleterious effects of the disease on bone calcium accretion during the critical years for BMC accrual. Participants with T1D have been found to have lower vitamin D levels in comparison to non-diabetic individuals [11] and have been shown to have an altered gastrointestinal microbiome [12] – both are factors that could contribute to impaired gastrointestinal calcium absorption. Hypercalciuria, another factor that could impair calcium availability, has also been described [13].

The objective of this study was to use a dual-stable calcium isotope method to investigate the effects of T1D on percent gastrointestinal calcium absorption and estimated calcium retention in a cohort of adolescent females. This is an established method for the assessment of calcium absorption that allows for comparison of the results in the current study to data obtained in healthy children from previously published studies conducted using the same protocol.

METHODS

Study design and participants

A group of 20 females aged 9–16 years recruited from the Pediatric Endocrinology Clinic at the University of Rochester (URMC) completed this cross-sectional study. Study visits occurred during summer and autumn months between 8/1/2015 and 12/1/2016. Inclusion

criteria included exposure to T1D for three years prior to enrollment, female sex, and Tanner stage 2 or greater breast development (as evaluated by physical examination). Exclusion criteria included presence of conditions predicted to affect calcium metabolism (chronic kidney disease, endocrine hormone deficiency, celiac disease), exposure to glucocorticoids (oral, parental, or inhaled), diuretics, or proton-pump inhibitor in the six months prior to enrollment, obesity (BMI of >99th percentile, to reduce the likelihood of including participants with type 2 diabetes), and time since menarche of greater than three years (to reduce the likelihood of including participants who had achieved peak bone mass). The study was approved by the Research Subjects Review Board (RSRB) at URM; informed consent and assent were obtained in all participants.

Study procedures

Stable calcium isotopes (^{42}Ca and ^{44}Ca) were obtained from Trace Sciences International (Wilmington, DE, USA) and prepared for human use by the specialty pharmacy AnazaoHealth Corporation (Tampa, FL, US). On the morning of the study, participants were admitted to the Clinical Research Center (CRC) at URM. Blood was obtained in the fasting state and a peripheral IV was placed. At the start of the absorption study, 2 mg of ^{42}Ca was administered intravenously over 5 minutes, followed by a 5mL flush with normal saline. With breakfast, participants consumed 30 mL milk (default) or calcium fortified orange juice (for participants who did not want milk) containing 7 mg ^{44}Ca which had been added to the beverage and refrigerated overnight. The glass containing the isotope was rinsed and the rinse was ingested to ensure the entire dose was received. The total amount of tracer administered was determined by pre- and post-weighing the dosing syringe. After administration of the oral isotope, a complete 24-hr urine collection was begun, with aliquots collected in 8-hr intervals. All participants remained in the CRC until completion of the urine collection.

Prior to the study visit, participants completed three-day dietary food records to estimate typical calcium intake. A 24-hr diet recall conducted by phone by a CRC nutritionist was used instead of 3-day diet records in four participants in whom the food records were found to be incomplete. Home dietary intake was used by the CRC nutritionist to devise a diet containing comparable calcium and caloric intake for the study period. All foods consumed during the study visit were provided by the CRC and weighed prior to consumption; uneaten food was again weighed. Calcium intake was determined from the food consumed using the Nutrition Data System for Research Software, version 2014 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

Isotope analysis and calculations

Urine samples were prepared for mass spectrometric analysis by precipitation with ammonium oxalate [14]. A calcium precipitate was isolated by centrifugation and ashed in a muffle furnace before being reconstituted in a 3% solution of ultrapure nitric acid and loaded onto a rhenium filament. Magnetic sector thermal ionization mass spectrometry (Triton TI mass spectrometer; ThermoQuest) was used to determine isotopic ratios of ^{42}Ca and ^{44}Ca to ^{43}Ca . The enrichment of each tracer in the urine samples was expressed as the percent excess (the degree to which the measured ratio was increased over the natural abundance

ratio). Calcium content of the urine samples was determined by atomic absorption spectrophotometry (Analyst 800 atomic absorption spectrophotometer, PerkinElmer).

Percent dietary calcium absorption was calculated as the ratio of the cumulative recovery of ^{44}Ca (oral tracer) to the cumulative recovery of ^{42}Ca (IV tracer) in the 24-hour urine sample. Cumulative recovery was calculated using the following equations, where 2.086% and 0.647% represent the natural abundances of the respective ^{44}Ca and ^{42}Ca tracers:

$$\text{Cumulative } ^{44}\text{Ca} = \sum (\Delta \text{ percent excess } ^{44}\text{Ca} * \text{total urine calcium} * 2.086\%) / ^{44}\text{Ca dose}$$

$$\text{Cumulative } ^{42}\text{Ca} = \sum (\Delta \text{ percent excess } ^{42}\text{Ca} * \text{total urine calcium} * 0.647\%) / ^{42}\text{Ca dose}$$

True calcium absorption (V_a) was calculated as the product of fractional calcium absorption and dietary calcium intake, where dietary calcium intake was the average of calcium intake at home and from the weighed-food record. Estimated calcium retention was calculated as V_a – the sum of measured 24-hr urine calcium excretion (V_u) and assumed endogenous fecal calcium excretion (V_{endo}), where V_{endo} was assumed to be 1.6 mg/kg/d based on findings from a study conducted in 25 children of similar age and dietary calcium intake [15]. The adequacy of the 24-hour period for assessment of calcium absorption outcomes has been previously demonstrated [16], and the equations outlined above including the use of assumed V_{endo} have been previously reported in pediatric studies [17–19].

Covariates

Height was assessed by wall-mounted stadiometer, weight by digital stand-on scale. Anthropometric measures were converted into Z-scores using US reference data [20]. Height velocity was assessed by review of clinical growth chart over the prior 12-months and reported as cm/yr. Pubertal status was determined by direct inspection by a pediatric endocrinologist [21]. T1D, fracture, and other medical history, and medication use were obtained by participant interview and verified by chart review. Laboratory measures were performed at UPMC and included hemoglobin A1c (HbA1c) assessed by high performance liquid chromatography, 25-hydroxyvitamin D assessed by liquid chromatography-tandem mass-spectrometry, and intact parathyroid hormone (PTH) assessed by electrochemiluminescent immunoassay. Bone-specific alkaline phosphatase (BSAP) and urinary n-telopeptides (NTX) were performed by quantitative chemiluminescent immunoassay at ARUP (Salt Lake City, UT, USA).

Statistical analysis

Data were analyzed using STATA 13 (StataCorp LP). Standard descriptive statistics were used to summarize participant characteristics and outcome variables. All variables were assessed for normality prior to analysis and t-tests or Wilcoxon rank sum tests were used to test group differences, as appropriate. Correlations were assessed using Pearson's product-moment coefficient or Spearman's rank correlation coefficient, as appropriate. Non-parametric variables were log-transformed prior to partial correlation analysis. Apparent

outlying data points seen on scatter plots were assessed graphically in STATA by a leverage-versus-residual-squared plot, and numerically for discrepancy (studentized residual $> |2|$), leverage (Hosmer and Lemeshow leverage $> 2k+2/n$), and influence [Cooks distance $> 4/n$ and DFBETA $> 2/\sqrt{n}$] [22]. Statistical significance was defined using a 2-sided p-value < 0.05 for all analyses.

RESULTS

Participant characteristics are provided in Table 1. The median age of participants was 14.2 years (range 9.1–16.5) with a median T1D duration of 5.6 years [interquartile range (IQR): 3.8–7.7]. The majority (80%) of participants used an insulin pump; the median total daily insulin dose was 0.9 units/kg/d (IQR: 0.8–1.2). The mean ($\pm$ SD) HbA1c on day of study visit was $9.0 \pm 1.5\%$, which was strongly correlated with average HbA1c over the previous 3 years; Pearson's $R=0.86$ ($p<0.001$). There were no reports of retinopathy, neuropathy, or microalbuminuria. One-quarter of participants (5/20) reported a history of fracture, the degree of impact associated with the fall was not recorded. Vitamin D deficiency [25-hydroxyvitamin D < 20 ng/mL (50 mmol/L)] and insufficiency [25-hydroxyvitamin D < 30 ng/mL (75 mmol/L)] was present in 20% and 55% of participants, respectively. Bone mineral related biochemistries are summarized in Supplemental Table 1. There were no significant associations between HbA1c and any of the bone biomarkers. All participants had serum bicarbonate levels in the normal range.

The outcomes from the calcium absorption studies for the entire cohort, stratified by pubertal status (Tanner Stage 2/3 versus 4/5) are provided in Table 2. Median dietary calcium intake at home and on the day of the study visit were similar [1304 mg/d (IQR: 869–1633) and 1166 mg/d (IQR: 908–1679)] and significantly correlated (Spearman's $\rho=0.91$, $p<0.001$). Calcium intake did not differ by pubertal status. Dietary calcium intake was lower than the recommended dietary allowance (RDA) of 1300 mg/day in 50% of participants [23]. Median percent calcium absorption and true calcium absorbed were 26.6 % (IQR: 18.9–35.6) and 277.9 mg/d (IQR: 246.9–417.7), respectively, and did not differ by pubertal status. Median urinary calcium excretion was greater for girls in late puberty [188.5 mg/d (IQR 119.0–207.5) or 2.9 mg/kg/d (IQR: 2.0–3.8)] compared to early puberty [89 mg/d (IQR: 61.5–104) or 1.8 mg/kg/d (IQR: 1.0–2.5)], $p=0.008$. Median estimated calcium retention was lower for girls in late puberty [21.3 mg/d (IQR: –17.5–132)] compared to early puberty [134.4 mg/d (IQR: 82.6–341.1)], $p=0.04$. There was no difference in percent calcium absorption between those who consumed the tracer in milk versus orange juice.

Five participants were found to have estimated calcium retention values of less than 0 (range –5 to –107 mg/d), all of whom were Tanner Stage 4 or 5. Participants with net negative calcium retention had greater median urinary calcium excretion [202.0 mg/d (IQR: 178.0–213.0) or 3.0 mg/kg/d (IQR: 2.7–4.0)] compared to those with net positive calcium retention [103.0 mg/d (IQR: 82.0–199.0) or 2.1 mg/kg/d (IQR: 1.2–3.0)], $p=0.02$, but were not different in dietary calcium intake, percent calcium absorption, or true calcium absorbed (Figure 1). In addition to being of more advanced pubertal stage, participants with net negative retention were older [15.7 years (IQR: 14.9–15.9)], had lower median height velocity [1.6 cm/yr

(IQR:1.6–1.7)], and lower median BSAP [20.2 $\mu\text{g/L}$ (IQR:19.4–22.9)] compared to those with net positive retention [13.3 years (IQR:11.2–15.0), 5.6 cm/yr (IQR:3.0–6.7), 53.3 $\mu\text{g/L}$ (IQR: 28.1–86.2)], $p=0.02$, 0.01, and 0.03, respectively. Diabetes related outcomes (HbA1c, insulin dose, disease duration) and urine NTX did not differ between those with net positive versus net negative calcium retention.

Two participants (10%) were found to have hypercalciuria as defined by 24-hr urine calcium above 4 mg/kg/d; this number doubled to four (20%) if a urine calcium/urine creatinine ratio (Uca/Ucr) of >0.22 was used to define hypercalciuria [24]. The relationship between 24-hr urine calcium and HbA1c is shown in Figure 2. A data point from one participant appeared to be an outlier and was found to have a large residual (studentized residual -2.8), high leverage (leverage=0.43) and high influence (Cooks $D=2.14$), as can be visualized by the distance and isolation from other data points. Excluding this outlier, there was a significant correlation between 24-hr urine calcium (mg/kg/d) and HbA1c (Pearson's r 0.55, $p=0.02$). If the outlying data were considered, the correlation was not significant (Pearson's r 0.23, $p=0.34$). Findings were similar if Uca/Ucr was used to assess calcium excretion or urine glucose was used to assess hyperglycemia. There was minimal attenuation of the correlation between urine calcium and HbA1c after adjustment for growth, true calcium absorbed, BSAP, or urine NTX (Table 3).

Percent calcium absorption was significantly inversely correlated with dietary calcium intake among participants consuming less than the RDA for calcium (Spearman's ρ -0.65 , $p=0.04$) but not for participants meeting the RDA (Spearman's ρ 0.2, $p=0.58$). Study outcomes in participants whose dietary intakes were above and below the RDA for calcium are shown in Supplemental Table 2. Those meeting the RDA had greater true calcium absorption, and trended towards a greater estimated calcium retention and lower percent calcium absorption. Estimated calcium retention was positively correlated with calcium intake (Spearman's ρ 0.49, $p=0.03$). The correlations between percent calcium absorption and estimated calcium retention and biomarkers of bone growth and mineral metabolism are shown in Table 4. Estimated calcium retention was significantly and positively associated with growth (height velocity, cm/yr), BSAP (bone formation marker) and urinary NTX (bone resorption marker); positive correlations observed between these variables and percent absorption did not reach statistical significance. There was no evidence of an association between either calcium absorption or estimated calcium retention with vitamin D or intact PTH. There was an inverse trend between HbA1c and percent calcium absorption (Pearson's r -0.39 , $p=0.08$) but this association was markedly attenuated when adjusted for dietary calcium intake (Pearson's r -0.28 , $p=0.25$). Adjustment for calcium intake did not attenuate or alter the significance of the observed correlations between calcium outcomes and growth or bone turnover markers.

Calcium absorption and retention outcomes from the present study were compared to previously published data by Abrams and colleagues [15, 17] in two studies performed in healthy girls of similar age that used the same dual-stable isotope approach. Compared to a sample of 13 healthy girls aged 9–15 years [17] with similar calcium intake, T1D participants had similar mean percent calcium absorption ($27.8\pm 8.9\%$ vs $24.5\pm 8.2\%$, $p=0.29$) and true calcium absorbed (348.4 ± 460 mg/d vs 322.1 ± 102.2 mg/d, $p=0.22$), but

greater urine calcium excretion (138.2 ± 64.1 mg/d vs 93.9 ± 43.8 mg/d, $p=0.04$). Compared to an earlier sample stratified by pubertal status [17], T1D participants in early puberty did not differ from healthy girls in any calcium outcomes. T1D girls in late puberty had similar mean percent calcium absorption (25.6 ± 7.9 % vs 25 ± 7.9 %), but higher mean calcium intake (1337.3 ± 479.9 mg/d vs 955 ± 230 mg/d, $p<0.01$), true calcium absorbed (325.8 ± 120.4 mg/d vs 234 ± 78 mg/d, $p=0.02$) and urinary calcium excretion (168.2 ± 134.7 mg/d vs 109 ± 58 mg/d, $p=0.01$).

DISCUSSION

To our knowledge, this is the first study to use a dual-stable isotope approach to evaluate calcium absorption and estimated calcium retention in adolescents with T1D. Our results did not identify an adverse effect of T1D on gastrointestinal calcium absorption, but did suggest that excess urinary calcium excretion during adolescence may contribute to whole body calcium loss during a time when bone mineral accretion is expected to be ongoing.

Bone mineral accretion begins *in utero*, peaks in adolescence, and continues until peak bone mass is attained in the late second or third decade of life [25]. One study found that females attained peak BMC at 18.8 years [26]; others have found that bone accrual in women continued into the third decade [27]. Because lower bone mass is associated with greater fracture risk [28], optimizing bone accrual and peak bone mass is a critical aspect of skeletal health [29]. Studies in children and adults with T1D have found deficits in bone mineral outcomes [5, 30], a finding which may contribute to the high fracture risk in this population.

The expected daily calcium retention necessary to promote bone mineral accrual during adolescence has been previously estimated. A study that used longitudinal DXA BMC data reported positive calcium retention for each year of life between 9–18 years in healthy girls; calcium retention ranged from 49.5 mg/d at age 17 years to 234.7 mg/d at age 13 years [31]. The average retention for early (9–13 years) and late (14–18 years) adolescence was 151 mg/d and 92 mg/d, respectively. A study that used stable isotopes in healthy girls similarly found that calcium retention was positive throughout adolescence, averaging 161 mg/d in early puberty and 44 mg/d in late puberty [17]. The estimated calcium retention values for T1D girls of 134 mg/d (early puberty) and 21 mg/d (late puberty) in the current study appeared lower than previously reported for healthy girls, although direct comparisons are limited without access to the primary data from earlier studies.

Notably, 40% of late pubertal T1D participants in this study had negative estimated calcium retention. All participants were under 18 years and within three years of menarche; and therefore would be expected to be accruing bone mineral and retaining calcium. In the context of previous studies that identified bone mineral deficits in T1D [4, 5], this result suggests insufficient calcium availability for bone mineralization as a possible mechanism underlying the finding of low BMD in patients with T1D. Alternatively, low calcium retention may be a marker of insufficient skeletal capacity to deposit calcium due to low bone formation. BSAP was lower among T1D participants in our study with negative estimated calcium retention; however the sample size limits our ability to fully account for the effects of growth and puberty on BSAP.

With the exception of one outlier who had high HbA1c and low urine calcium, there were strong correlations between measures of hyperglycemia (HbA1c and urine glucose) and urine calcium. The outlier was of Black race; previous studies have shown that Black girls have lower urine calcium excretion compared to Whites [32, 33]. Contrary to previous reports, there was no relationship between urine calcium and urine sodium in our study [34]. Depending on the definition used, between 10–20% of T1D participants in this study had clinical hypercalciuria. The prevalence of clinical hypercalciuria in healthy children has been reported to be between 5–10% [35]. The reported prevalence of hypercalciuria in T1D is higher, ranging from 20–50% of participants; [13, 36] although these data were collected over two-decades ago when tight glycemic control in T1D was less common. The standard clinical definitions of hypercalciuria are not physiologically derived, and an inverse relationship between urine calcium excretion and BMC has been shown in children without clinical hypercalciuria [37], suggesting that even modest increases in urine calcium loss may be detrimental to skeletal health. The etiology of excess urinary calcium excretion in T1D is unclear. We found that the relationship between hyperglycemia and urinary calcium was only minimally attenuated after adjusting for calcium absorption or bone turnover. We therefore speculate that hyperglycemia results in excess urinary calcium excretion through impaired renal calcium reabsorption. Potential mechanisms to be explored in future studies include an effect of hyperglycemia on PTH physiology [38] or an osmotic diuresis related to glucosuria. Of note, sodium-glucose co-transporter-2 (SGLT2) inhibitors, which increase urinary glucose excretion, have recently been approved for use in patients with type 2 diabetes. A study in mice showed that SGLT2 therapy was associated with increased urinary calcium excretion [39]; there is currently insufficient data in humans to determine if these drugs are associated with changes in urinary calcium, bone mineralization, or fracture risk.

Our study found no evidence of an adverse effect of T1D or hyperglycemia on calcium absorption. This finding is similar to that of a previous study in adults with diabetes that also found no difference in calcium absorption compared to healthy controls; retention was not reported [40]. The inverse correlation observed between dietary calcium intake and percent absorption in our study was similar to what has been described in other populations [15, 19], and suggests that the gastrointestinal tract is able to respond to low calcium intake with increased absorption.

One-half of participants did not meet the RDA for calcium. Data from the SEARCH for Diabetes in Youth Study showed that almost three-quarters of T1D adolescents met the RDA for calcium [41]; however intake was assessed by a food frequency questionnaire, a method that may overestimate calcium intake. Calcium intake was positively correlated with retention in our study, suggesting that a nutritional evaluation coupled with dietary education to improve calcium intake may be a feasible intervention to increase calcium retention in calcium deficient T1D patients. However, given the potential risk for hypercalciuria and associated complications, caution should be used to avoid excessive calcium supplementation.

This study has limitations. Estimates of V_{endo} were based on values previously obtained in healthy children. Empiric determination of V_{endo} requires prolonged stool collections (seven days or more) and higher calcium isotope dosing and is therefore both costly and

burdensome for participants. V_{endo} has not previously been determined in T1D adolescents. None of the participants in this study had known gastrointestinal disorders that might be expected to alter V_{endo} , although the possible effect of hyperglycemia is unknown. DXA scans were not obtained so we were unable to evaluate associations between calcium absorption, retention, and BMC. We did not include a contemporaneous control population, although the study was conducted using the same protocol previously used in healthy children. Given the large number of factors that influence urinary calcium excretion, larger sample sizes are needed to completely evaluate the relationship between hyperglycemia, growth and urine calcium. Finally, generalizability is limited to females.

In summary, our study suggests that calcium retention may be diminished in adolescent girls with T1D as a result of excess urinary calcium loss. Further studies are needed to determine if insufficient calcium availability for BMC accrual is a contributor to the deficient BMD and increased fracture risk known to be associated with T1D. This information is needed to design interventions to support optimal bone calcium deposition in this pediatric population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We evaluated calcium absorption and retention in girls with type 1 diabetes
- One quarter of girls had negative estimated calcium retention
- Girls with negative calcium retention had greater urinary calcium excretion
- Urinary calcium excretion was positively associated with hyperglycemia
- The effect of urinary calcium loss on bone mineralization needs to be explored

Figure 1.

Comparison of calcium outcomes including (A) 24-hour urine calcium excretion, (B) dietary calcium intake, (C) percent calcium absorption, and (D) true calcium absorbed between female T1D participants with negative estimated calcium retention (n=5) versus those with positive estimated calcium retention (n=15). Only 24-hour urine calcium excretion differed significantly between the groups. Wilcoxon rank sum test used to compare calcium outcomes between groups; statistical significance was defined as two-sided p-value <0.05. Solid circle in 1C is an outside data point (>1.5*interquartile range)

Figure 2.

Scatter plot showing the relationship between 24-hour urine calcium excretion and hemoglobin A1c in 20 female T1D participants. Dashed line represents the estimated regression line including all data points ($R^2=0.23$, $p=0.34$). Solid line represents the estimated regression line excluding data point in lower right corner as an outlier ($R^2=0.55$, $p=0.02$), outlier determination based upon studentized residual = -2.8 , leverage = 0.43 (where $2k=2/n=0.3$), Cook's $D = 2.1$ (where $4/n=0.2$), $DFBETA = -2.3$ (where $2/\text{Sqrt}(n)=0.45$). Pearson's product-moment coefficient used to determine strength of relationship between variables; statistical significance was defined as two-sided p-value <0.05 .

Table 1

Participant characteristics

n	20
Age, y	14.2 (9.1–16.5) ¹
T1D duration, y	5.6 (3.8–7.7) ²
Hemoglobin A1c, %	9.0 (8.1–9.6)
History of DKA, n	9 (45) ³
Insulin dose, units/kg/d	0.9 (0.8–1.2)
Insulin pump, n	16 (80)
BMI Z-score	1.0 (0.8–1.3)
Height Z-score	0.5 (–0.3–0.8)
Height velocity, cm/y	3.2 (1.7–6.1)
Tanner Stage	
2–3	7 (35)
4–5	13 (65)
Fracture history, n	5 (25)
25(–OH)D, ng/mL	28.6 (20.8–30.8)
25(–OH) D, nmol/L	71.1 (51.9–76.9)
25(–OH)D < 20 ng/ml (50 nmol/L), n	4 (20)
24-hr urine calcium, mg/kg/d	2.4 (1.6–3.3)
24-hr urine calcium > 4 mg/kg/d, n	2 (10%)

¹ Median (range)² Median (interquartile range), all such values not otherwise marked³ n (%), all such values

Table 2

Indices of calcium absorption and retention in adolescent girls with T1D

	Entire sample	Early pubertal ¹	Late pubertal ¹
n	20	8	12
Age, y (range)	14 (9–16)	11 (9–13)	15 (13–16)
Calcium intake, mg/d	1278.5 (888.5–1653.5) ²	1351.8 (887.8–1655.8)	1251.5 (888.5–1628.5)
Percent calcium absorption, %	26.6 (18.9–35.6)	32.3 (22–039.3)	25 (18.9–31.3)
True calcium absorbed, mg/d	277.9 (246.9–417.7)	294.5 (219.8–571.9)	277 (246.9–376.3)
Urinary calcium, mg/d	119.0 (89.0–200.0)	89.0 (61.5–104.0)	188.5 (119.0–207.5) **
Estimated calcium retention, mg/d	82.6 (–0.1–193.4)	134.4 (82.6–341.4)	21.3 (–17.5–132.0) *
Negative calcium retention, n	5	0	5 *

¹ Early puberty defined as Tanner Stage 2 or 3, late puberty as Tanner Stage 4 or 5² Median (interquartile range), all such values

* Significantly different from early pubertal, p<0.05

** Significantly different from early pubertal, p<0.01

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Table 3

Partial correlations between urinary calcium and hemoglobin A1c in 20 adolescent girls with T1D after adjustment for growth, gastrointestinal calcium absorption or bone turnover

	24-hr urine calcium	p	24-hr urine calcium	p	24-hr urine calcium	p	24-hr urine calcium	p
HbA1c	0.55	0.02	0.57	0.02	0.52	0.03	0.55	0.02
Height velocity ²			-0.14	0.59				
True calcium absorbed ¹					0.32	0.27		
Urine NTX ²							0.16	0.53
BSAP ²							-0.18	0.49

¹ Analyses exclude data for one participant determined to be an outlier. Urine calcium expressed as mg/kg/d
² Log transformed
BSAP, bone-specific alkaline phosphatase (formation marker); HbA1c, hemoglobin A1c; NTX, n-telopeptides (resorption marker)

Table 4

Associations between percent calcium absorption and estimated calcium retention and markers of growth and bone mineral metabolism in 20 adolescent girls with T1D

		Percent Calcium Absorption		Estimated Calcium Retention	
		p		p	
Height velocity ^{1,2}	0.35 ²	0.13	0.47	0.04	
Bone-specific alkaline phosphatase	0.37	0.10	0.61	0.01	
Urine collagen cross-linked n-telopeptide	0.39	0.15	0.51	0.02	
25(-OH)D	-0.05	0.83	-0.28	0.25	
1,25(-OH) ₂ D	-0.15	0.54	0.19	0.40	
Intact PTH	0.15	0.51	0.04	0.88	

¹ expressed as cm/y

² Spearman's rank correlation coefficient reported