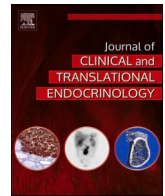




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Original research

Bone metabolism and incretin hormones following glucose ingestion in young adults with pancreatic insufficient cystic fibrosis

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ABSTRACT

Background: Gut-derived incretin hormones, including glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1), regulate post-prandial glucose metabolism by promoting insulin production. GIP, GLP-1, and insulin contribute to the acute bone anti-resorptive effect of macronutrient ingestion by modifying bone turnover. Cystic fibrosis (CF) is associated with exocrine pancreatic insufficiency (PI), which perturbs the incretin response. Cross-talk between the gut and bone ("gut-bone axis") has not yet been studied in PI-CF. The objectives of this study were to assess changes in biomarkers of bone metabolism during oral glucose tolerance testing (OGTT) and to test associations between incretins and biomarkers of bone metabolism in individuals with PI-CF.

Methods: We performed a secondary analysis of previously acquired blood specimens from multi-sample OGTT from individuals with PI-CF ages 14–30 years (n = 23). Changes in insulin, incretins, and biomarkers of bone resorption (C-terminal telopeptide of type 1 collagen [CTX]) and formation (procollagen type I N-terminal propeptide [P1NP]) during OGTT were computed.

Results: CTX decreased by 32% by min 120 of OGTT (P < 0.001), but P1NP was unchanged. Increases in GIP from 0 to 30 mins ($\rho = -0.48$, P = 0.03) and decreases in GIP from 30 to 120 mins ($\rho = 0.62$, P = 0.002) correlated with decreases in CTX from mins 0–120. Changes in GLP-1 and insulin were not correlated with changes in CTX, and changes in incretins and insulin were not correlated with changes in P1NP.

Conclusions: Intact GIP response was correlated with the bone anti-resorptive effect of glucose ingestion, represented by a decrease in CTX. Since incretin hormones might contribute to development of diabetes and bone disease in CF, the "gut-bone axis" warrants further attention in CF during the years surrounding peak bone mass attainment.

Introduction

Bone health tracks strongly throughout the growing years and into adulthood, and disruption of normal bone accrual during childhood might increase risk for osteoporosis and fracture in later life [1]. Myriad behavioral and biological factors contribute to peak bone mass,

particularly nutrition and related hormonal mediators [2–4]. The biological mechanisms through which nutrition impacts bone health are not clearly defined.

Bone accretion largely depends on the independent and coordinated actions of the bone-forming osteoblasts and the bone-resorbing osteoclasts [5]. These bone-regulating cells are responsive to a variety of

Abbreviations: CF, cystic fibrosis; PI, pancreatic insufficiency; OGTT, oral glucose tolerance test; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; CTX, C-terminal telopeptide of type 1 collagen; P1NP, procollagen type I N-terminal propeptide.

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stimuli, including macronutrient ingestion [6,7]. One hypothesized mechanism through which nutrition might influence peak bone mass is through activation and/or deactivation of cells orchestrating bone turnover, thereby leading to modifications in bone accrual [8]. For example, ingestion of a glucose-containing solution (75 g) yielded an approximately 50% reduction in bone resorption as measured via C-terminal telopeptide of type 1 collagen (CTX) [7,9–11]. Others have reported similar effects of glucose ingestion on bone resorption, and that glucose-related effects on bone are more pronounced following oral glucose administration compared to routes of glucose administration that bypass the gastrointestinal tract (e.g., intravenous administration) [10,11]. These collective findings suggest that carbohydrate ingestion has a bone anti-resorptive effect, and that gut-mediated mechanisms are likely involved.

Several hormonal mediators of nutrient metabolism are hypothesized to contribute to this “gut-bone axis.” Following macronutrient ingestion, numerous gut-derived hormones are mobilized to regulate satiety, gastric motility, and metabolism [12]. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are the main gut-derived hormones or “incretins” that play an integral role in augmenting insulin secretion [13]. *In vitro* studies suggest that the bone-forming osteoblasts and bone-resorbing osteoclasts possess membrane-bound receptors for GIP, GLP-1, and insulin, and experimental studies in humans indicate that these incretins, as well as insulin, promote increases in bone formation and decreases in bone resorption [14–18]. Accordingly, medical conditions with perturbed incretin and/or insulin response to nutrient ingestion might threaten bone health through modifications of the “gut-bone axis.”

This study evaluates the “gut-bone axis” in a sample of emerging and young adults with PI-CF, a genetic condition associated with compromised pulmonary health, impaired nutritional status, and diabetes [19], as well as reduced bone mass and increased fracture risk [20,21]. PI and poor incretin response to food intake are suspected to contribute to CF-related diabetes (CFRD) [22–24], but might also contribute to CF-related bone disease [20,21]. To interrogate how carbohydrate ingestion may influence bone turnover in people with PI-CF, we evaluated changes in biomarkers of bone metabolism following ingestion of an oral glucose solution and tested the relationships of changes in insulin and incretin hormones with biomarkers of bone metabolism. Based on previous studies in adults [10,11,13,25,26], we hypothesized that in adolescents and young adults with PI-CF, bone resorption (assessed via CTX) would decrease significantly following glucose ingestion, but that bone formation (assessed via procollagen 1 intact *N*-terminal propeptide [P1NP]) would not be affected. Additionally, we hypothesized that GIP, GLP-1, and insulin responses following glucose ingestion are associated with reductions in bone resorption as indicated by greater reductions in CTX.

Methods

Study design and participants

We performed a secondary analysis of data and biological specimens collected during a prospective study designed to test the underpinnings of post-glucose load hypoglycemia in people with PI-CF [27]. Insulin secretory rates and glucagon results from the multi-sample oral glucose tolerance tests (OGTT) conducted in 23 youth and young adults (39% female) ages 14–30 years receiving treatment for CF at the Children’s Hospital of Philadelphia or University of Pennsylvania Medical Center were previously published [27]. A more detailed description of study participants, protocols, and procedures were published previously [27].

The diagnoses of CF and PI were confirmed using clinical records documenting sweat testing and/or cystic fibrosis transmembrane regulatory mutation analysis, and pancreatic enzyme replacement therapy and/or fecal elastase levels <200 µg/g. Exclusion criteria included history of organ transplantation, CFRD with fasting hyperglycemia (fasting

glucose \geq 126 mg/dL), and systemic glucocorticoid therapy within previous four weeks of the study visit.

Anthropometry

Standing height and weight were assessed using a wall-mounted stadiometer and electronic scale, respectively. Body mass index (BMI; kg/m²) was calculated.

Oral glucose tolerance test

All participants completed an OGTT following an overnight fast. Participants ingested a solution containing 1.75 g of glucose per kg of body weight (75 g of glucose maximum) over a 5-minute period, indicating time point “0.” Using an indwelling intravenous catheter, blood specimens were collected at mins –10, –1, 10, 20, 30, and every subsequent 15 mins until 240 mins. For subjects that experienced symptomatic hypoglycemia with glucose < 65 mg/dL, or glucose \leq 50 mg/dL in the absence of symptoms, testing was terminated early. For the present analyses, only data from mins 0 to 120 are included.

Blood biochemistries

At each time point indicated above, glucose, insulin, GIP, and GLP-1 were assayed in duplicate. Glucose was measured using the YSI 2300 glucose analyzer (Yellow Spring Instruments, Yellow Spring, OH) and, for immediate results, the Nova StatStrip glucometer (Data Sciences International, St. Paul, MN). These glucose assessment methods demonstrate strong agreement with one another [27]. Insulin was measured in duplicate using a double antibody radioimmunoassay [28], and active GLP-1 and total GIP were measured in duplicate by ELISA (Millipore, Billerica, Massachusetts). Biomarkers of bone metabolism, CTX and P1NP, were assessed at time points 0, 30, 60, and 120. CTX was evaluated using the Cobas e411 automated analyzer (Roche Diagnostics International Ltd., Basel, Switzerland), and P1NP was evaluated using a commercially available ELISA kit (Antibodies-Online Inc., Limerick, PA).

Calculations

Percent changes (% Δ) in CTX, P1NP, GIP, GLP-1, insulin, and glucose were calculated. Percent change in CTX and P1NP from mins 0 to 120 were computed and were abbreviated as CTX-% $\Delta_{0-120\text{min}}$ and P1NP-% $\Delta_{0-120\text{min}}$, respectively. Percent change in GIP, GLP-1, insulin, and glucose were calculated for mins 0 to 30, 0 to 120, and 30 to 120. As an example, % Δ in GIP from mins 0 to 30 was abbreviated as GIP-% $\Delta_{0-30\text{min}}$. Percent change was calculated as follows: ((measurement 2 – measurement 1) / measurement 1) \times 100. Using data from all available time points, incremental area under the curve (iAUC) was calculated for GIP, GLP-1, insulin, and glucose for mins 0 to 30 and mins 0 to 120 using the trapezoidal method. iAUCs for CTX and P1NP were calculated for mins 0 to 120. As an example, iAUC for CTX and insulin from mins 0 to 120 are abbreviated as CTX-AUC_{0-120mins} and insulin-AUC_{0-120mins}, respectively.

Statistical analyses

Prior to conducting statistical analyses, all data were visually inspected for outliers, biologically implausible data points, and non-normal distributions. Subject descriptive characteristics are summarized using mean (standard deviation) or median (inter-quartile range) for continuous variables and count (percent) for categorical variables.

To evaluate change in glucose, insulin, incretins, and biomarkers of bone turnover during OGTT, mixed regressions were performed. For each analysis, min 0 of the OGTT was used as the comparison time point. Relationships between incretins, metabolic parameters, and age with

biomarkers of bone turnover during OGTT were evaluated using non-parametric Spearman's rank correlation. We also compared % Δ and iAUC for biomarkers of bone metabolism during OGTT between males and females using linear regression.

Various sensitivity analyses were performed. Linear regression was used to assess associations between changes in insulin/incretins and biomarkers of bone metabolism during OGTT while accounting for min 0 values of insulin/incretins. Additional linear regression analyses were performed to assess age and sex interactions in the association between changes in insulin/incretins and biomarkers of bone metabolism during OGTT.

All statistical analyses were performed using STATA (version 15). P-values < 0.05 were considered statistically significant.

Results

Descriptive characteristics

Descriptive characteristics of the study participants were previously reported [27]. Their age ranged from 14.6 to 30.6 years, with an average age of 24.6 ± 4.4 years, and there was a greater proportion of males compared to females (57 % male) and Whites compared to Blacks (96 % White). The BMI of study participants ranged from 17.8 to 27.8 kg/m², with an average BMI of 22.4 ± 2.7 kg/m². Nine percent of participants had normal glucose tolerance (n = 2; 1-hour glucose \leq 155 mg/dL and 2-hour glucose < 140 mg/dL), 35 % had early glucose intolerance (n = 8; 1-hour glucose \geq 155 mg/dL and 2-hour glucose < 140 mg/dL), 43 % had impaired glucose tolerance (n = 10; 2-hour glucose 140–199 mg/dL), and 13 % had diabetes (n = 3; 2-hour glucose \geq 200 mg/dL).

Changes in incretins and metabolic outcomes during OGTT

Changes in glucose, insulin, GLP-1, and GIP during OGTT in the total study sample are presented in Fig. 1. Glucose, insulin, GLP-1, and GIP increased during OGTT (all P < 0.001). Glucose and insulin increased until mins 60 and 90, respectively, and declined thereafter. GLP-1 and GIP increased similarly from mins 0 to 30. While GIP remained increased from baseline until min 120, GLP-1 returned to baseline by min 120.

CTX and P1NP during OGTT are displayed in Fig. 2. CTX decreased significantly during OGTT (P < 0.05). CTX at mins 30 (P = 0.39) and 60 (P = 0.050) were not significantly different than min 0, but CTX at min 120 was significantly lower than min 0 (P = 0.004). When expressed in units of % Δ , CTX decreased significantly between mins 0 and 30 (P = 0.004), 0 and 60 (P < 0.001), and 0 and 120 (P < 0.001). From mins 0 to 120, CTX decreased by an average of 32%, ranging from approximately -5% to -62%. P1NP responses varied from mins 0 to 120, ranging from approximately +66% to -33%, with a mean change of about +1%.

Correlations between incretin and metabolic responses and biomarkers of bone turnover during OGTT

Bivariate correlations between % Δ changes in incretins and metabolic parameters and CTX during OGTT are presented in Table 1. GIP-% $\Delta_{0-30\text{min}}$ was negatively correlated with CTX-% $\Delta_{0-120\text{min}}$, suggesting that individuals with greatest increases in GIP from mins 0 to 30 had the greatest reductions in CTX from mins 0 to 120 (Fig. 3). GIP-% $\Delta_{30-120\text{min}}$ was positively correlated with CTX-% $\Delta_{0-120\text{min}}$, suggesting that individuals with the greatest decreases in GIP from mins 30 to 120 had the greatest reductions in CTX from mins 0 to 120. GLP-1-% $\Delta_{30-120\text{min}}$ was negatively correlated with CTX-% $\Delta_{0-120\text{min}}$, but this relationship did not meet significance (P = 0.061; Supplemental Fig. 1). Insulin-% $\Delta_{30-120\text{min}}$ was positively correlated with CTX-% $\Delta_{0-120\text{min}}$, but this relationship was not significant (P = 0.105; Supplemental Fig. 2). Percent change in glucose, insulin, and incretins were not correlated with P1NP during OGTT (data not shown).

Bivariate correlations between iAUCs for incretins and metabolic

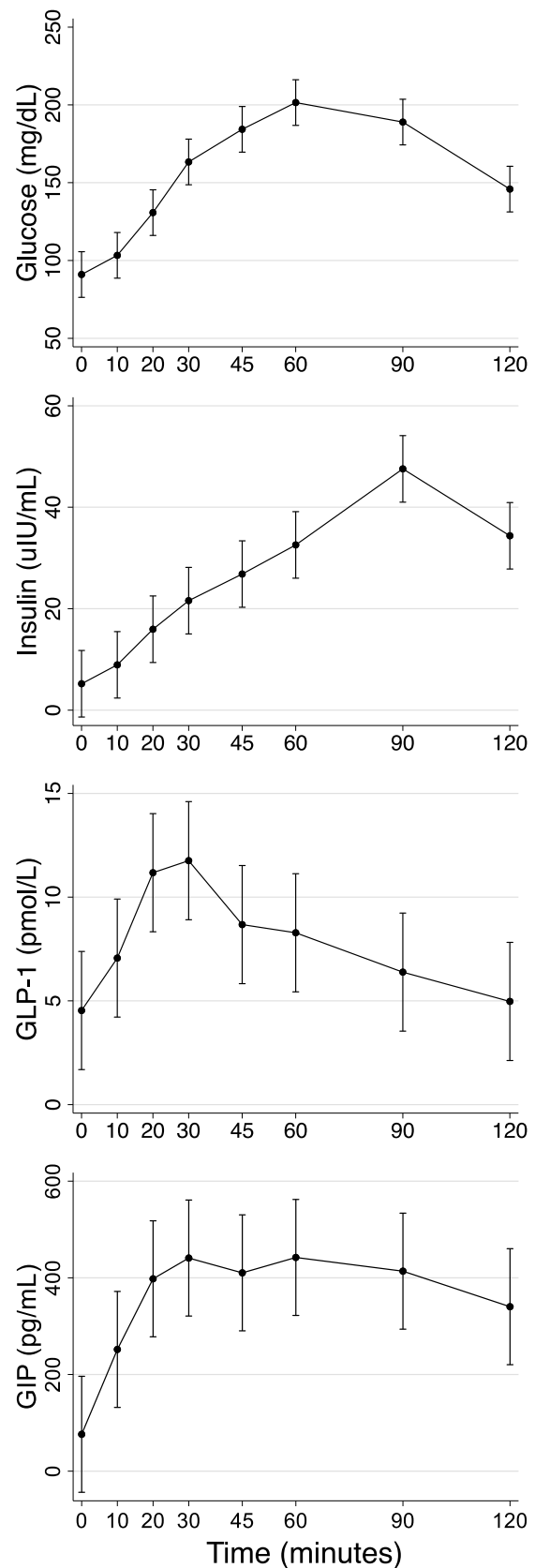


Fig. 1. Changes in glucose, insulin, GLP-1, and GIP during OGTT in youth with PI-CF. Vertical bands represent the 95% confidence interval. OGTT, oral glucose tolerance test; CF, cystic fibrosis.

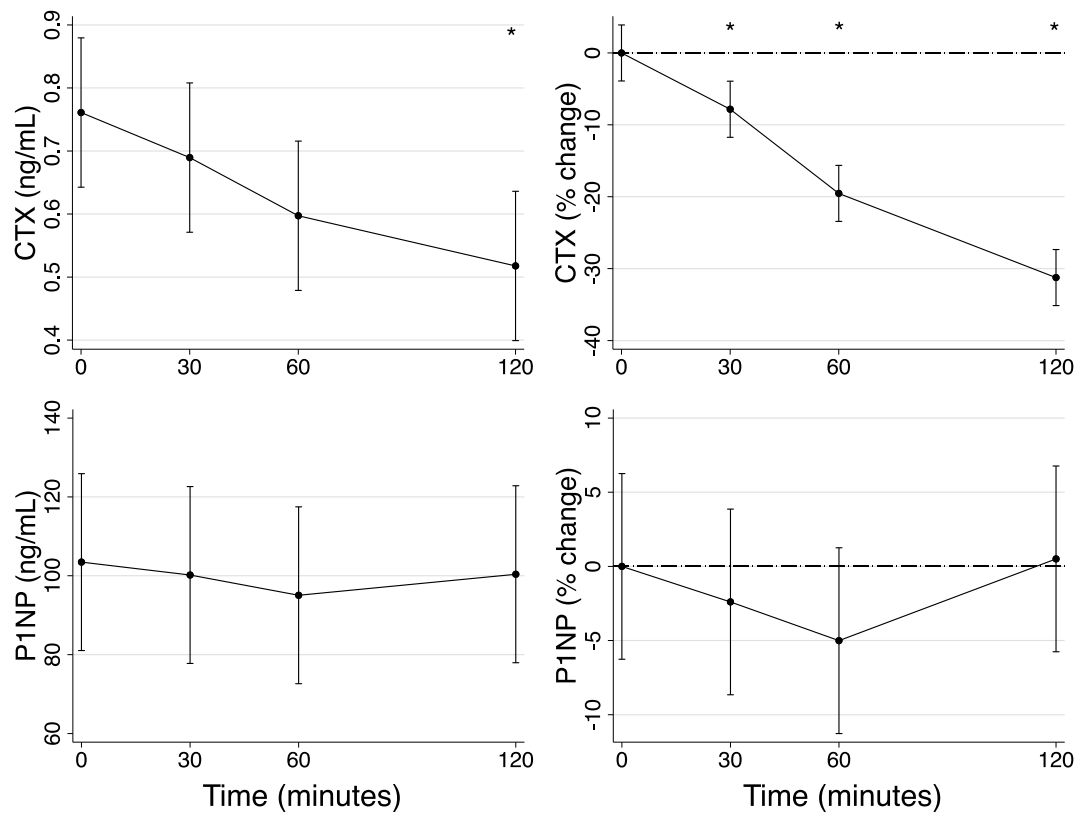


Fig. 2. CTX (top) and P1NP (bottom) % Δ during OGTT in youth with PI-CF. Vertical bands represent the 95% confidence interval. *Significantly different than minute 0. % Δ , percent change; CTX, C-terminal telopeptide of type 1 collagen.

Table 1

Associations between % Δ in incretin and metabolic parameters and CTX (minutes 0–120) at varying timepoints during OGTT.

	Time (minutes)	Spearman's rho	P
GIP	0–30	−0.54	0.008
	0–120	−0.16	0.471
	30–120	0.50	0.016
GLP-1 ^a	0–30	−0.25	0.268
	0–120	0.06	0.805
	30–120	0.40	0.061
Glucose	0–30	−0.14	0.517
	0–120	−0.03	0.879
	30–120	−0.01	0.964
Insulin	0–30	−0.16	0.452
	0–120	0.15	0.506
	30–120	0.35	0.105

% Δ , percent change; CTX, C-terminal telopeptide of type 1 collagen; OGTT, oral glucose tolerance test; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1.

^a n = 22.

parameters and CTX during OGTT are presented in **Supplemental Table 1**. Insulin-iAUC₀₋₃₀ was negatively correlated with CTX-iAUC_{0-120min}, but this relationship was not statistically significant ($P = 0.063$). Glucose, GIP, and GLP-1 iAUCs were not correlated with CTX-iAUC_{0-120min}. Glucose, insulin, GIP, and GLP-1 iAUCs were also not significantly correlated with P1NP-iAUC_{0-120min} (data not shown).

Correlations between age and sex with biomarkers of bone turnover during OGTT

Age correlated positively with CTX-% Δ _{0-120min} ($\rho = 0.34$, $P = 0.105$) and CTX-iAUC_{0-120min} ($\rho = 0.40$, $P = 0.061$), but relationships were not significant. Age did not correlate with changes in P1NP during

OGTT (data not shown). Additionally, CTX-iAUC_{0-120min} differed between males and females (males: -22.9 ± 12.6 , females: -8.9 ± 6.7 ; $P = 0.005$), and this difference persisted after adjusting for age ($P = 0.011$). Changes in P1NP during OGTT did not differ between males and females.

Sensitivity analyses

To determine whether relationships between changes in insulin, GIP, and GLP-1 and changes in CTX and P1NP were confounded by min 0 values of insulin or incretin hormones, linear regression analyses were performed. Associations remained consistent when including min 0 values for the respective predictor variable (insulin, GIP, or GLP-1) as an additional regression model parameter (data not shown). Additionally, no age or sex interactions with insulin, GIP, or GLP-1 were found in the relationships of these hormones with bone turnover markers (data not shown).

Discussion

The “gut-bone axis” is a hypothesized contributor to nutrition and diabetes-related effects on bone [3,7,13,29]. Experimental studies suggest that consumption of macronutrients results in acute anti-resorptive effects on bone, and that hormones involved in nutrient metabolism, such as the gut-derived incretins GIP and GLP-1, as well as insulin contribute to these effects [9,30–32]. Cystic fibrosis is associated with a unique form of diabetes that involves deranged incretin and insulin responses to macronutrient consumption [33,34]. Deficits in bone density and increased fracture risk are reported in patients with CF [35,36]; thus, invoking disruption to the “gut-bone axis” as a plausible contributor to CF related bone disease. The current study addresses these gaps in knowledge by evaluating the “gut-bone axis” in a sample of adolescents and young adults with PI-CF. Similar to prior studies in healthy

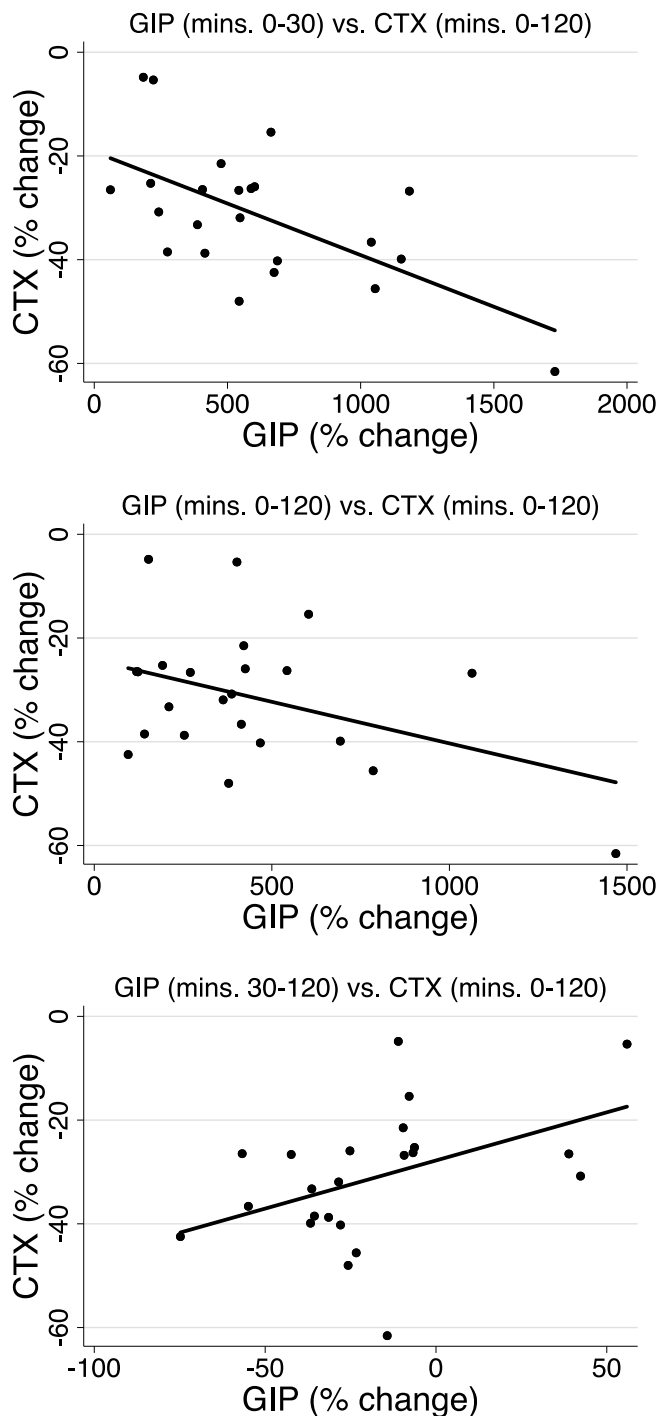


Fig. 3. Associations between % Δ in GIP and CTX during OGTT in youth with PI-CF. % Δ , percent change; CTX, C-terminal telopeptide of type 1 collagen.

adults [10,13,25,26], our data suggest that acute glucose ingestion results in a significant decrease in CTX, a biomarker of bone resorption, but did not have an effect on P1NP, a biomarker of bone formation. Our data also suggest that the incretin hormones, notably GIP, might contribute to these changes in bone metabolism following glucose ingestion. Interpretation of our findings is limited by the lack of a healthy control group and individuals with pancreatic sufficient CF. The pathophysiology of CFRD includes a perturbed incretin and insulin response to nutrient intake [37,38]. These data support the need for future research involving the “gut-bone axis” in the convergence of diabetes and bone disease in CF.

Previous data suggest that the bone forming osteoblasts and bone resorbing osteoclasts are responsive to consumption of macronutrients [39], resulting in a shift in the balance between bone formation and resorption, predominantly by decreasing bone resorption [29]. Consumption of a fixed amount of carbohydrate (75 g of glucose) in adults results in a significant reduction in CTX, a well-characterized biomarker of bone resorption, by 120 mins following ingestion [7,9,10]. We also found significant decreases in CTX following glucose ingestion in our study, as demonstrated by a median CTX-% $\Delta_{0-120\text{min}}$ of about -32% by min 120, which is lesser in magnitude than the approximately 50% reduction in CTX by min 120 of OGTT that has been consistently reported previously in healthy adults [7,9,10]. Other studies comparing bone metabolism following macronutrient ingestion have reported suppressed effects on bone resorption in adults with hypothyroidism, nonalcoholic fatty liver disease, and type 2 diabetes compared to healthy controls [10,25,40]. Together, these studies suggest that the biological responses of bone to macronutrient and/or food consumption might be modified in individuals with chronic health conditions that exert more global effects on metabolism.

Our study sample included adolescents and young adults with PI-CF, a condition which leads to impaired nutrient metabolism and nutritional status [41]. Endocrine pancreatic dysfunction is also common in people with CF, and patients with CF are at increased risk for developing diabetes [19]. CF-related diabetes is at least in part attributed to progressive pancreatic β -cell functional decline resulting in abnormal insulin secretion, including a diminished early-phase insulin response followed by a pronounced late-phase insulin response [27]. In addition to dysglycemia, the perturbed insulin response might also impact bone health since insulin inhibits osteoclastogenesis [42], which might contribute to decreased bone resorption during insulin infusion, as reported in a mouse model of type 1 diabetes [43]. In the current study, late-phase insulin was marginally associated with changes in CTX, suggesting that bone-augmenting effects of insulin might be perturbed in youth with the greatest risk of developing CFRD. The metabolic health status among our study sample was variable, ranging from normal glucose tolerance to diabetes. The small sample size prevented comparisons across groups based on glucose control.

Our data suggest a potential role of incretins in decreasing bone resorption, as a greater increase in GIP from mins 0 to 30 and a greater decrease in GIP from mins 30 to 120 was associated with more pronounced reductions in CTX. In contrast, greater decreases in GLP-1 from mins 30 to 120 were only marginally associated with decreases in CTX. These data are consistent with findings of others that infusion of GIP and/or GLP-1 significantly decreases bone resorption [13,26], and that GIP and GLP-1 receptor knockout mice have decreased bone mass [15,44]. ‘Incretin mimetic’ drugs (e.g., GLP-1 receptor agonists) increase insulin and decrease glucagon production in the pancreas, and ‘dipeptidyl peptidase-4 (DPP-4) inhibitors’ reduce degradation of incretin hormones, GIP and GLP-1, by the DPP-4 enzyme [45]. While insulin therapy is the first-line treatment for CFRD [46], recent data suggest a potential benefit of incretin-based therapies on insulin secretion following a meal [47]. Incretin-based therapies are suspected to benefit bone health [48], but effects on the gut-bone axis have not yet been explored in CF.

Most studies evaluating acute effects of nutrient ingestion on bone metabolism reported decreases in bone resorption, but inconsistent responses in bone formation [10,11,13,25,26,30,49]. Our data are consistent with these studies; change in P1NP following glucose ingestion was highly variable. In fact, most studies evaluating bone formation via P1NP reported null effects [10,11,13,25,26], as did other studies utilizing alternate bone formation markers such as osteocalcin [11,49,50]. Extended experimental protocols and inclusion of complementary biomarkers of bone formation should be considered in future studies.

Few studies address age or sex-related differences in acute changes in bone metabolism following macronutrient ingestion [51,52]. Bone

modeling, which involves an uncoupling of bone formation and resorption, is dominant during the years preceding peak bone mass, whereas bone remodeling, which involves a coordination between bone formation and resorption, is dominant in adulthood. Thus, biological effects of macronutrient ingestion on bone are likely dependent on both age and sex. We only found a marginal and not statistically significant association of younger age with greater decreases in CTX following glucose ingestion, and that males had a significantly greater CTX iAUC compared to females. CF-related complications such as diabetes and bone disease increase with age, and perhaps these complications involve the gut-bone axis. Further, bone metabolism occurs at a more accelerated rate in childhood and adolescence compared to young adulthood [52], suggesting that the gut-bone axis is amplified during the growing years and might contribute to sex differences in bone density and fracture.

Strengths and limitations

This study used previously acquired data and blood specimens to investigate a novel biological mechanism that likely contributes to nutrition and diabetes effects on bone health in a vulnerable population during the years surrounding peak bone mass attainment. Although the original study included a healthy control group to compare against individuals with PI-CF [27], blood specimens for assessment of incretins and bone biomarkers were not available from the healthy control group for the current study. This represents a main limitation of this study. Additionally, all participants had exocrine PI necessitating pancreatic enzyme replacement therapy. PI is among the most common complication of CF, occurring in upwards of 85% of patients [53]. To understand the contribution of maldigestion resulting from PI, future studies in people with PI-CF should also consider including individuals with normal pancreatic function. Moreover, about half of our sample had reactive hypoglycemia during the OGTT [27], which might have contributed to the wide variation in bone responses to glucose ingestion. Coupled with the small sample size, the variability in incretin, insulin, and bone responses to glucose ingestion likely limited our statistical power.

The OGTT method used in this study has both strengths and limitations. Our use of a standardized 75 g OGTT, performed following an overnight fast, helped minimize confounding resulting from nocturnal and daily variation in metabolic response to food ingestion, and also helped facilitate comparisons with previously published studies that followed a similar approach [7,9–11]. Rather than ingesting single nutrients in isolation, humans consume mixtures of foods and nutrients while engaging in normal activities such as sleep, exercise, and sedentary behavior. Specific to CF, high-fat diets are often recommended to ensure adequate caloric intake [54]. Future studies involving the gut-bone axis in CF should apply translatable experimental approaches (e.g., high-fat mixed meal tolerance tests) while encompassing normal nocturnal and daily variation in components of the gut-bone axis.

Conclusions

This study provides novel insights into the role of nutrition on influencing peak bone mass and the development of CF-related bone disease in adolescents and young adults with PI-CF. We report significant decreases in bone resorption following glucose ingestion, and a potential underlying role of incretin hormones and insulin in these antiresorptive effects. Although our study is limited by the lack of a healthy control group, these novel data give insight into potential mechanisms linking complex co-morbidities in CF, namely diabetes and bone disease. Since people with CF typically consume a high-fat diet that might contribute to these developments, the impact of habitual dietary intake on the gut-bone axis warrants consideration. Furthermore, future studies should seek to define the “normal” post-prandial changes in bone metabolism during the years surrounding peak bone mass, notably with respect to age, sex, race, and puberty.

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Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcte.2022.100304>.

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