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Bone resorption and incretin hormones following glucose ingestion in healthy emerging adults

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ABSTRACT

Background: Studies in adults indicate that macronutrient ingestion yields an acute anti-resorptive effect on bone, reflected by decreases in C-terminal telopeptide (CTX), a biomarker of bone resorption, and that gut-derived incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), facilitate this response. There remain knowledge gaps relating to other biomarkers of bone turnover, and whether gut-bone cross-talk is operative during the years surrounding peak bone strength attainment. This study first, describes changes in bone resorption during oral glucose tolerance testing (OGTT), and second, tests relationships between changes in incretins and bone biomarkers during OGTT and bone micro-structure.

Methods: We conducted a cross-sectional study in 10 healthy emerging adults ages 18–25 years. During a multi-sample 2-hour 75 g OGTT, glucose, insulin, GIP, GLP-1, CTX, bone-specific alkaline phosphatase (BSAP), osteocalcin, osteoprotegerin (OPG), receptor activator of nuclear factor kappa-β ligand (RANKL), sclerostin, and parathyroid hormone (PTH) were assayed at mins 0, 30, 60, and 120. Incremental areas under the curve (iAUC) were computed from mins 0–30 and mins 0–120. Tibia bone micro-structure was assessed using second generation high resolution peripheral quantitative computed tomography.

Results: During OGTT, glucose, insulin, GIP, and GLP-1 increased significantly. CTX at min 30, 60, and 120 was significantly lower than min 0, with a maximum decrease of about 53 % by min 120. Glucose-iAUC$_{0\text{-}30}$ inversely correlated with CTX-iAUC$_{0\text{-}120}$ (rho = -0.91, $P < 0.001$), and GLP-1-iAUC$_{0\text{-}30}$ positively correlated with BSAP-iAUC$_{0\text{-}120}$ (rho = 0.83, $P = 0.005$), RANKL-iAUC$_{0\text{-}120}$ (rho = 0.86, $P = 0.007$), and cortical volumetric bone mineral density (rho = 0.93, $P < 0.001$).

Conclusions: Glucose ingestion yields an anti-resorptive effect on bone metabolism during the years surrounding peak bone strength. Cross-talk between the gut and bone during this pivotal life stage requires further attention.

Introduction

Peak bone mass is achieved around the third decade of life [1], setting the stage for lifelong bone health. Nutrition is a main modifiable factor involved in peak bone mass attainment [2], and endocrine mediators of nutrient metabolism are purported to contribute to these effects [3,4]. The "entero-insulin axis," for example, involves cross-talk between the gut and the pancreas for regulation of post-prandial macronutrient metabolism [5]. Following food ingestion, K and L cells of the gastrointestinal tract release glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), respectively, which signal the pancreatic beta cells to promote insulin secretion and pancreatic alpha cells to decrease glucagon production in a glucose-dependent manner [6]. Beyond their well-defined actions in glucose control, these gut-derived hormones, referred to as incretins, also regulate bone turnover [7,8].

The integral cellular machinery involved in bone metabolism, osteoblasts and osteoclasts, undergo dynamic changes in activity to regulate bone formation and bone resorption across sleep and wake periods. These bone-regulating cells, which possess membrane-bound receptors for GIP and GLP-1 [9–12], are also responsive to acute bouts of food/nutrient ingestion. Clinical studies in adults report greater decreases in

Abbreviations: OGTT, oral glucose tolerance test; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; CTX, C-terminal telopeptide of type 1 collagen; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor kappa-β ligand; PTH, parathyroid hormone; BSAP, bone-specific alkaline phosphatase.

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bone resorption, measured via CTX, following oral vs intravenous glucose administration, despite a similar glycemic response [13–15]. Intravenous infusion and subcutaneous injection of incretin hormones results in a bone anti-resorptive effect [15–19]. These collective findings support a gut-mediated mechanism underpinning nutrition effects on bone. Bone biology during the years surrounding peak bone mass attainment is unique to that of the adult skeleton [20]. Bone modeling, a process involving the independent action of the osteoblasts and osteoclasts to enhance bone size, mass, and strength, is dominant in adolescence [21], whereas bone remodeling, a process involving the coordinated action of the osteoblasts and osteoclasts to maintain mineral homeostasis, is dominant in the ageing skeleton [22]. To this point, clinical studies involving incretin hormones and bone metabolism (i.e., the "gut-bone axis") have primarily focused on adults. As such, studies in individuals experiencing the adolescent-to-adult transition are required to confirm that the gut-bone axis is operative during the important life stage of peak bone mass and peak bone strength attainment.

The Bone Health and Osteoporosis Foundation (BHOF), formerly the National Osteoporosis Foundation, sponsored a 2016 summary statement on lifestyle factors in peak bone mass [23]. This report highlighted the importance of nutrition in peak bone mass attainment and identified critical knowledge gaps in this field of study. Notably, clinical studies defining the intermediary biological mechanisms in nutrition effects on bone and focused on the adolescent to young adult transition were highlighted as critically needed areas of pursuit. To address these needs, we conducted a cross-sectional study in 10 healthy adolescents and young adults ages 18 to 25 years. Our primary aim was to determine normal changes in bone resorption during 2-hour 75 g multi-sample oral glucose tolerance testing (OGTT). OGTT was used to streamline comparisons to prior studies that followed similar approaches [13,14,16,17]. The primary outcome of interest was CTX, which is a biomarker of bone resorption that has been reported in numerous adult studies describing bone anti-resorptive effects of glucose ingestion [13–17,24–27]. Based on these previous studies, our *a priori* hypothesis was that CTX would decrease significantly by min 120 of OGTT. Earlier clinical studies involving the gut-bone axis have mainly focused on CTX and procollagen 1 intact *N*-terminal propeptide (P1NP) as biomarkers of bone resorption and formation, respectively. For this reason, the extent to which other biomarkers of bone metabolism and/or bone-derived factors are responsive to OGTT is unclear. [28,29]. Our secondary aims were to determine relationships between glucose, insulin, and incretin hormones and 1) changes in biomarkers of bone turnover during OGTT and 2) measures of cortical and trabecular bone morphology assessed via second generation high resolution peripheral quantitative computed tomography (HR-pQCT).

Methods

Study design and participants

We enrolled a sample of 10 healthy adolescents and young adults to participate in this cross-sectional study. This desired sample size was based on previously published results from our team [30] and others [16,17,25], indicating that a sample size of n = 10 would provide >90 % power to observe an approximately 50 % decrease in CTX between mins 0 and 120 of OGTT.

Subjects were ages 18 to 25 years, without chronic diseases or growth disorders, and had a self-reported body mass index (BMI; kg/m^2) in the 'healthy weight' range. Healthy weight status was based on age-specific cutoffs using BMI-for-age percentile for individuals ages 18 to 19 years [31] and BMI for individuals for individuals ages 20–25 years [32]. Potential subjects were excluded if they recently sustained a fracture or were taking medications known to influence bone metabolism. Subjects participated in two laboratory visits. The OGTT was held at the UGA Clinical and Translational Research Unit, whereas the questionnaires, anthropometric measurements, DXA, and HR-pQCT were completed at the UGA Nutrition and Skeletal Health Laboratory. Both laboratory visits were completed within 22 days of one another. Prior to participating in the study, all subjects provided written informed consent. The Institutional Review Board for Human Subjects at The University of Georgia approved all study protocols and procedures.

Anthropometry

Standing height and weight were measured using a wall-mounted stadiometer and digital scale, respectively. BMI was calculated, and for subjects <20 years of age, BMI-for-age percentile was calculated [33]. All anthropometric measurements were performed in triplicate and averaged by a single trained researcher.

Oral glucose tolerance test

Subjects completed a multi-sample 2-hour OGTT on the morning following an overnight fast. A fasting blood specimen was collected (min 0), at which point subjects were instructed to drink a beverage containing 75 g of glucose (Trutol) over a period of 10 min. Using an indwelling intravenous catheter, additional blood specimens were collected at mins 30, 60, and 120. Serum samples were collected using tubes pre-treated with EDTA, and plasma samples were collected using tubes pre-treated with protease inhibitors.

Blood biochemistries

Glucose, insulin, total GIP, active GLP-1, CTX, BSAP, osteocalcin, osteoprotegerin (OPG), receptor activator of nuclear factor kappa-β ligand (RANKL), sclerostin, and parathyroid hormone (PTH) were assayed at mins 0, 30, 60, and 120 of OGTT. Glucose, CTX, and BSAP assays were performed at Athens-Piedmont Medical Center, and insulin, GIP, GLP-1, osteocalcin, OPG, RANKL, sclerostin, and PTH were assayed at the University of Georgia College of Veterinary Medicine Cytometry Core. Serum glucose was measured via spectrophotometry using a Beckman Coulter AU5800 clinical chemistry analyzer (Beckman Coulter, Brea, CA). Serum CTX and BSAP were assayed via immunoassay using the Roche Cobas 602 (Roche Diagnostics, Basel, Switzerland) and Beckman Coulter DxI 800 (Beckman Coulter, Brea, CA), respectively. Insulin, GIP, and GLP-1 were assessed in duplicate via a magnetic bead-based multiplex platform (Millipore, HMHEMAG-34-K). Osteocalcin, OPG, sclerostin, and PTH were assessed in duplicate via a magnetic bead-based multiplex platform (Millipore, HBNMAG-51K), and RANKL was assessed using a single plex assay (Millipore, HRNKLMAG-51K).

Calculations

Data from mins 0, 30, 60, and 120 for each outcome of interest were used to calculate incremental area under the curve (iAUC) for each measure. As an example, iAUC from mins 0 to 120 for CTX is abbreviated as CTX-iAUC$_{0\text{-}120}$. iAUCs capturing the 'early phase' response (OGTT mins 0–30) were also calculated. As an example, iAUC from mins 0 to 30 for GLP-1 is abbreviated as GLP-1-iAUC$_{0\text{-}30}$.

Dual-energy X-ray absorptiometry

Total body (less head), lumbar spine (L1-L4 vertebrae), and non-dominant forearm dual-energy X-ray absorptiometry (DXA) scans were performed using a Hologic Horizon densitometer (Hologic, Inc.). Scans were performed and analyzed by a single trained research assistant using APEX software version 2.1. In our lab, total body BMD and lumbar spine BMD showed strong reliability in n = 32 healthy adults (CVs < 1 %). BMD Z-scores were computed using published reference ranges from the Bone Mineral Density in Childhood Study [34]. Since these reference ranges terminate at the age of 20 years, subjects >20 years of age were assigned the age of 20 for Z-score calculations.

High resolution peripheral quantitative computed tomography

The Scanco XtremeCT II HR-pQCT scanner (SCANCO Medical AG) was used for assessment of tibia cortical and trabecular bone characteristics (Fig. 1). A single trained research assistant performed and analyzed all scans. First, lower leg length was measured using a sliding caliper from the distance of the medial malleolus to the tibial plateau. Measurements were completed on the non-dominant leg, determined by asking the subject which leg they would use to kick a soccer ball. Next, a scout view scan was completed. The reference line was manually placed at the proximal edge of the distal end plate. Scans were acquired at a fixed offset distance (22.5 mm proximal to the reference line), as previously described [35], and at a relative offset distance (30 % relative to the reference line). A series of 168 parallel slices were collected, using a 10.2 mm image stack and 61 mm isotropic voxel size, centered at the fixed and 30 % sites proximal to the reference line. At the fixed site, total volumetric BMD (Tt.vBMD), trabecular area (Tb.Ar), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and the bone volume to total volume fraction (BV/TV) were assessed. At the 30 % site, cortical volumetric BMD (Ct.vBMD), cortical area (Ct.Ar), cortical thickness (Ct.Th), intra-cortical porosity (Ct.Po), and cortical pore diameter (Ct.Po.Dm) were assessed. Following scan acquisition, the quality of each scan was graded from a scale of 1 (excellent quality) to 5 (poor quality) using the method described by Whittier et al. [35]. A priori, it was determined that only scans that received a score of 1 to 3 would be included in final analyses. In our lab, tibia trabecular and cortical bone measures showed strong reliability in n = 6 healthy adults. With the exception of Ct.Po (CV = 5.8 %), all CVs were <1 %.

Statistical analyses

Statistical analyses were performed using STATA version 15. All data were visually inspected for outliers and biologically implausible data points, which were subsequently excluded from the dataset prior to conducting analyses. Descriptive characteristics were summarized using mean/standard deviation for continuous variables, and count (percentage) for categorical variables.

Changes in biomarkers of bone metabolism, incretin hormones, insulin, and glucose during OGTT were evaluated using linear mixed-effects regression ("mixed" command in STATA). Separate analyses were performed for each outcome of interest. For each analysis, min 0 was used as the reference time point against which subsequent time points were compared. Spearman rank order correlation was used to assess associations between iAUCs for biomarkers of bone metabolism, incretins, insulin, and glucose, and bone outcomes from DXA and HR-pQCT. All analyses described above were repeated while excluding the two male subjects to eliminate potential confounding of sex. For all analyses, P-values <0.05 were considered statistically significant.

Results

Descriptive characteristics

Descriptive statistics are presented in Table 1. The study sample included 80 % female (n = 8) and 10 % Black (n = 1), with an average age of about 22 years and an average BMI of about 23 kg/m^2. All subjects had a fasting glucose <100 mg/dL and a 2-hour glucose <140 mg/dL, indicating normal glucose control as defined by the American Diabetes Association [36].

Changes in insulin, incretins, and bone biomarkers during OGTT

Changes in glucose, insulin, and incretins during OGTT are presented in Fig. 2 and changes in bone biomarkers during OGTT are presented in Fig. 3. Glucose, insulin, GIP, and GLP-1 increased significantly during OGTT and reached a peak at min 30. Whereas glucose and GLP-1 returned to min 0 values by min 120, insulin and GIP at min 120 remained greater than min 0. With respect to biomarkers of bone metabolism, only CTX changed significantly during OGTT. CTX at min 30 (P = 0.011), 60 (P < 0.001), and 120 (P < 0.001) was significantly

Table 1
Participant characteristics.

	Mean ± SD
Age, years	21.8 ± 1.7
Female, n (%)	8 (80)
White, n (%)	8 (80)
Height, cm	165.4 ± 8.0
Weight, lb	140.0 ± 21.0
BMI, kg/m^2	23.2 ± 3.1
Total body BMD, Z-Score	−0.50 ± 1.3
Lumbar spine BMD, Z-Score	−0.29 ± 1.3
1/3 radius BMD, Z-Score	0.02 ± 1.3
Glucose, mg/dL[a]	81.3 ± 6.1
2-hour glucose, mg/dL	92.6 ± 15.7
Insulin, pg/mL[a]	1066.7 ± 1462.5
GIP, pg/mL[a]	84.1 ± 41.2
GLP-1, pg/mL[a]	4.6 ± 3.5
CTX, pg/mL[a]	491.6 ± 130.3
BSAP, mcg/L[a]	8.9 ± 1.8
Osteocalcin, pg/mL[a]	25803.1 ± 17367.9
OPG, pg/mL[a]	394.4 ± 184.1
RANKL, pg/mL[a]	135.6 ± 116.5
Sclerostin, pg/mL[a]	2173.5 ± 824.1
PTH, pg/mL[a]	61.02 ± 24.7

BMI, body mass index; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; CTX, C-terminal telopeptide, BSAP, bone-specific alkaline phosphatase; OPG, osteoprotegerin; RANKL, nuclear factor kappa-β ligand; PTH, parathyroid hormone. [a]Fasting measure from min 0 of OGTT.

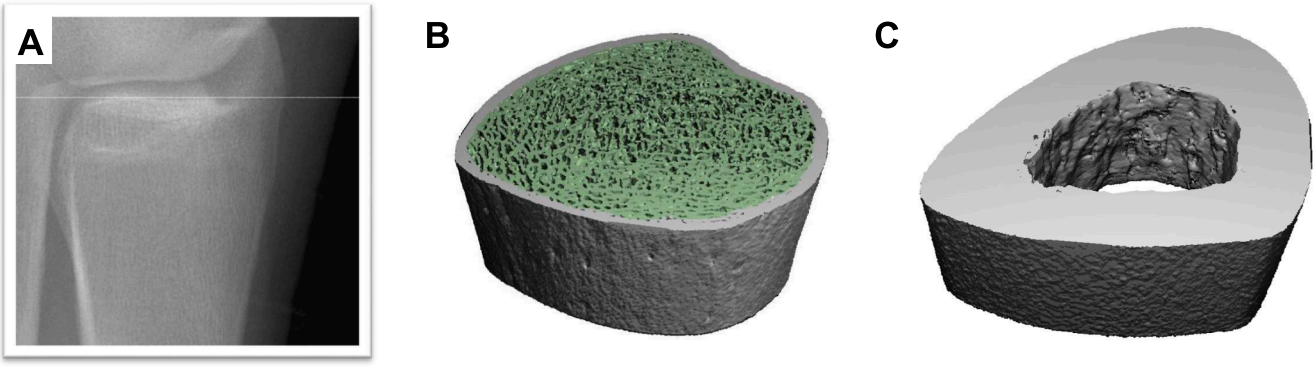

Fig. 1. "Scout view" scan showing reference line placement (for the 22.5 mm scan region; A) and reconstructed 3-dimensional images of trabecular (B) and cortical (C) bone regions.

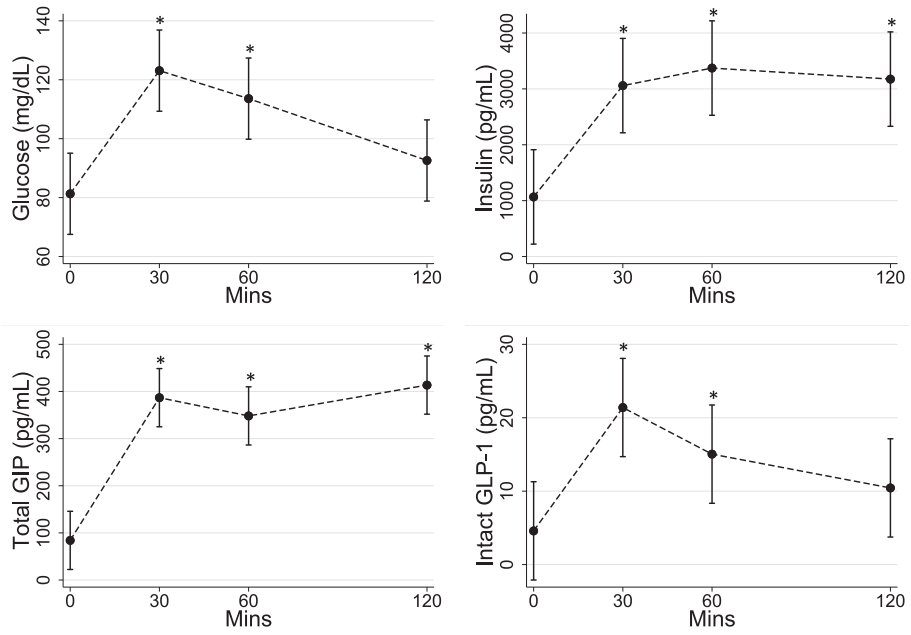

Fig. 2. Changes in glucose, insulin, total GIP, and intact GLP-1 during OGTT in healthy emerging adults. Error bars indicate standard error. *Significantly different than min 0 ($P < 0.05$).

lower than min 0. By min 30, 60, and 120, CTX decreased by approximately 20 %, 30 %, and 53 % compared to min 0, respectively. Sensitivity analyses excluding the two male subjects revealed similar changes in CTX during OGTT. By mins 30, 60, and 120, CTX decreased by approximately 19 % ($P = 0.030$), 36 % ($P < 0.001$), and 52 % ($P < 0.001$) compared to min 0, respectively, when excluding the two male subjects.

Correlations between incretins and bone biomarkers during OGTT

Bivariate correlations between glucose, insulin, incretins, and bone biomarkers were assessed using Spearman rank correlation (Table 2). Glucose-iAUC$_{0\text{-}30}$ was inversely correlated with CTX-iAUC$_{0\text{-}120}$ (Fig. 4). GLP-1-iAUC$_{0\text{-}30}$ was positively correlated with BSAP-iAUC$_{0\text{-}120}$ and RANKL-iAUC$_{0\text{-}120}$ (Fig. 5). When excluding the two male subjects, the inverse correlation between glucose-iAUC$_{0\text{-}30}$ and CTX-iAUC$_{0\text{-}120}$ (rho = -0.905, $P = 0.002$) and the positive correlation between GLP-1-iAUC$_{0\text{-}30}$ and RANKL-iAUC$_{0\text{-}120}$ (rho = 0.829, $P = 0.0416$) remained significant, but the correlation between GLP-1-iAUC$_{0\text{-}30}$ and BSAP-iAUC$_{0\text{-}120}$ was not significant (rho = 0.643, $P = 0.119$).

Correlations between incretins and HR-pQCT bone outcomes

Overall, HR-pQCT scans were of high quality. For the tibia trabecular bone region (22.5 mm from the distal end plate), five scans received a grade of 1 and three scans received a grade of 2. One scan received a grade of 4 and was excluded from analyses. For the 30 % tibia, eight scans received a grade of 1 and two scans received a grade of 2.

Spearman correlations between glucose, insulin, GIP, and GLP-1 iAUCs and bone outcomes from DXA and HR-pQCT are presented in Supplemental Table 1. Glucose, insulin, and GIP iAUCs did not correlate with DXA or HR-pQCT bone measures. However, GLP-1-iAUC$_{0\text{-}30}$ was positively correlated with Ct.vBMD (rho = 0.93, $P < 0.001$; Fig. 6). After excluding the two male subject, the association between GLP-1 iAUC and Ct.vBMD remained significant (rho = 0.89, $P = 0.007$).

Discussion

This study fills important knowledge gaps relating to the gut-bone axis during the critical years of peak bone mass and peak bone strength attainment [13–17,24–27,37–39]. Our results reveal that glucose ingestion yields a rapid, acute decrease in bone resorption, as indicated by a significant reduction in CTX. Although other biomarkers of bone metabolism did not change significantly during OGTT, GLP-1 response correlated with changes in BSAP and RANKL during OGTT and with tibia Ct.vBMD assessed via HR-pQCT. The current study is the first to support a bone anti-resorptive effect of glucose ingestion and potential involvement of incretin hormones in emerging adults. While these results align closely with prior studies in older adults [13–17,24–27], they also help to expand our current knowledge on the involvement of incretin hormones in peak bone strength.

The primary aim of this study was to assess changes in bone resorption during OGTT. In agreement with our *a priori* hypothesis, CTX, which is a biomarker of bone resorption [29], decreased significantly by min 120 of OGTT. Whereas a decrease in CTX is consistent with numerous previously published studies in healthy adults [13–17,24–27], as well as a recently published study in individuals ages 14 to 30 years with pancreatic insufficient cystic fibrosis (CF) [30], the current study is the first to report these effects in healthy adolescents and young adults, which coincides with the typical period of peak bone mass and peak bone strength attainment. We observed a ~53 % decrease in CTX by min 120 of OGTT, which is comparable to prior studies in healthy adults that report a relatively consistent ~50 % decrease in CTX by min 120 of OGTT [13–17,25–27]. Since this study was not designed to compare effects of glucose ingestion on bone metabolism at varying stages across the lifespan, future adequately powered studies are warranted to address this knowledge gap.

In contrast to the well-characterized associations between glucose ingestion and bone resorption [13–17,24–27], effects on bone formation are less clear. While some studies reported that a standard 75 g OGTT significantly decreased P1NP [24,25], a common biomarker of bone formation [40], others reported that bone formation remains unchanged [14,15,17,27]. In our study, we did not assess P1NP since numerous previous studies have consistently reported null associations during 2-

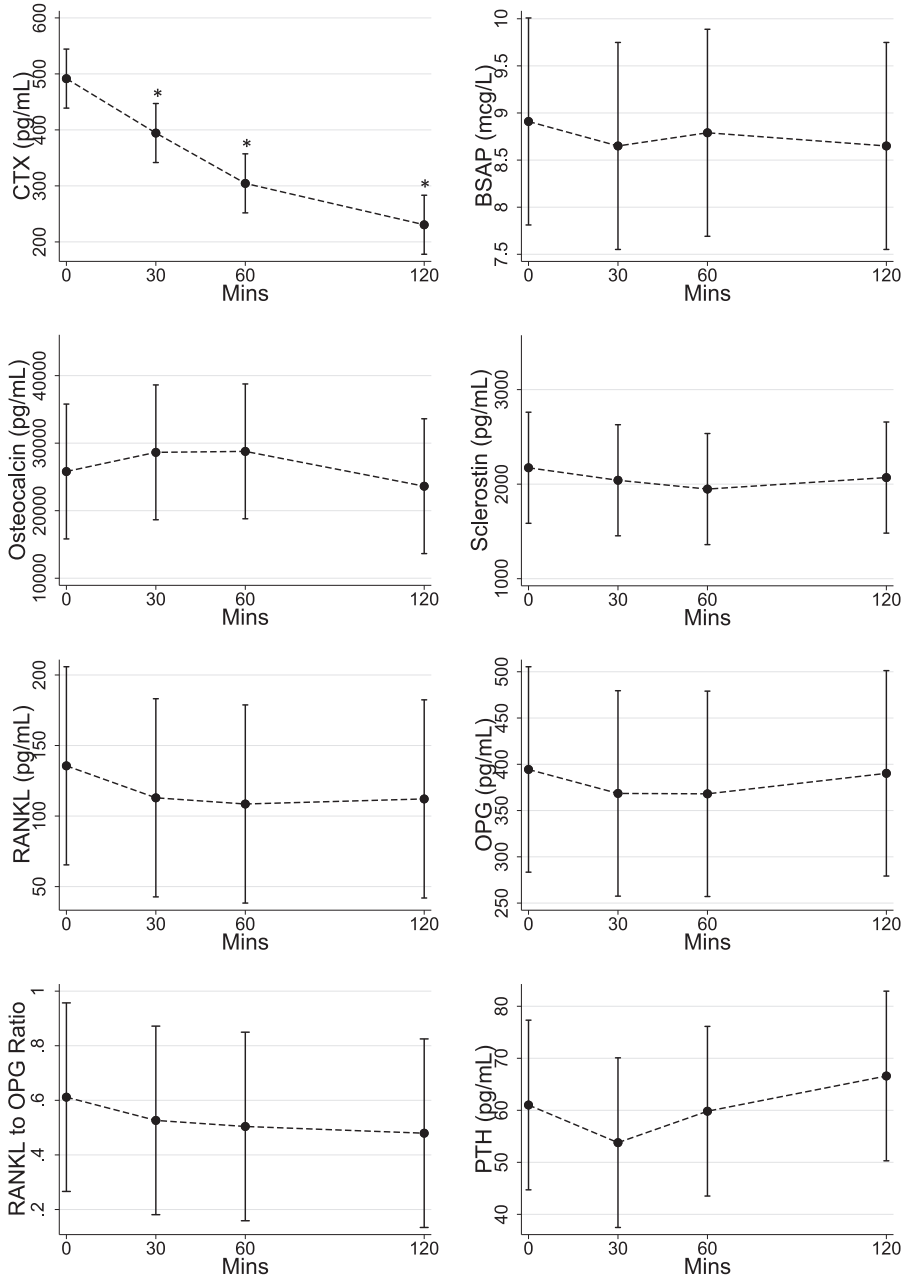

Fig. 3. Changes in biomarkers of bone metabolism during OGTT in healthy emerging adults. *Significantly different than min 0 ($P < 0.05$).

Table 2
Spearman correlation between iAUCs for glucose, insulin, GIP, and GLP-1 from minutes 0–30 and biomarkers of bone metabolism from minutes 0–120.

	Glucose		Insulin		GIP		GLP-1	
	Rho	P	Rho	P	Rho	P	Rho	P
CTX	−0.91	<0.001	−0.43	0.244	−0.14	0.701	0.58	0.099
BSAP	−0.41	0.243	0.12	0.765	0.56	0.090	0.83	0.005
OPG	0.21	0.555	0.20	0.606	0.41	0.244	0.35	0.356
Osteocalcin	0.16	0.651	0.10	0.798	0.13	0.726	0.00	1.000
Sclerostin	0.09	0.815	0.40	0.286	0.62	0.054	0.35	0.356
PTH	0.06	0.868	−0.03	0.932	−0.18	0.627	0.13	0.732
RANKL	−0.63	0.070	0.12	0.779	0.17	0.668	0.86	0.007
RANKL to OPG ratio	−0.12	0.779	0.38	0.352	−0.33	0.420	−0.12	0.779

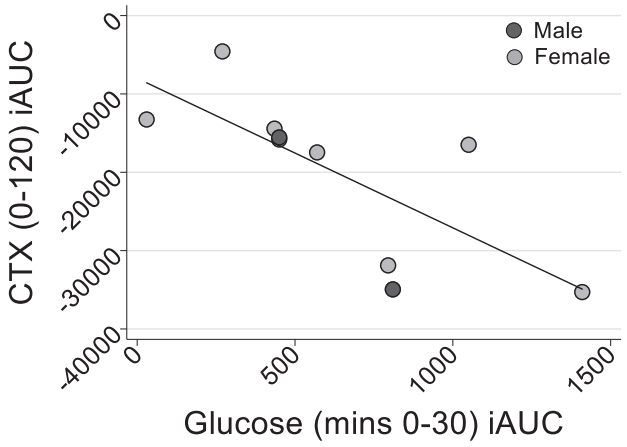

Fig. 4. Association between glucose-iAUC$_{0\text{-}30}$ and CTX-iAUC$_{0\text{-}120}$ in healthy emerging adults. Black dots are for males and gray dots are for females.

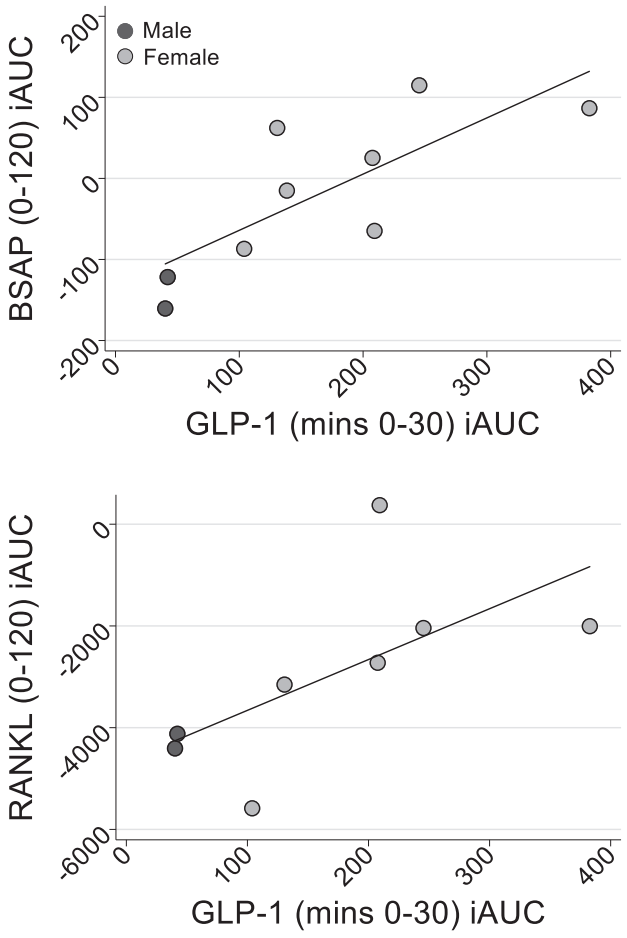

Fig. 5. Association between GLP-1-iAUC$_{0\text{-}30}$ and BSAP-iAUC$_{0\text{-}120}$ (top) and RANKL-iAUC$_{0\text{-}120}$ (bottom) in healthy emerging adults. Black dots are for males and gray dots are for females.

hour OGTT [14,15,17,25,27]. Rather, we evaluated BSAP and total osteocalcin as biomarkers of bone formation, which were unchanged during OGTT. Since CTX was our primary outcome of interest, we might not have had sufficient statistical power to observe effects on other bone outcomes.

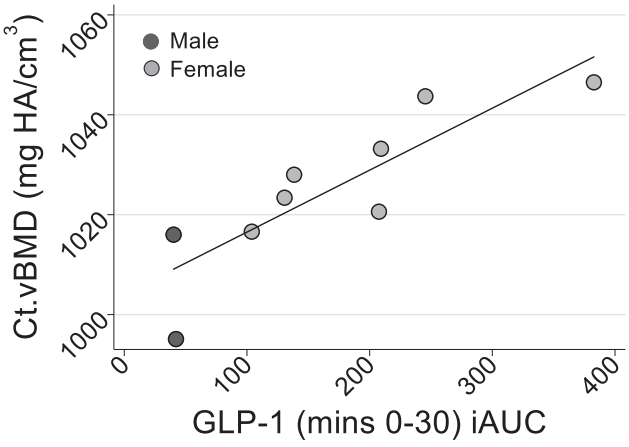

Fig. 6. Association between GLP-1-iAUC$_{0\text{-}30}$ and Ct.vBMD in healthy emerging adults.

Gut-derived incretin hormones, GIP and GLP-1, are proposed regulators of bone metabolism following macronutrient ingestion [15,37,41,42]. Preclinical studies have demonstrated that osteoclasts and osteoblasts express GIP and GLP-1 receptors, and that binding of GIP and GLP-1 to these receptors inhibit bone resorption and promote bone formation [6,12,43]. Clinical studies administering exogenous GIP and/or GLP-1 via subcutaneous injection or intravenous infusion consistently report decreases in bone resorption, but mostly null effects on bone formation [15–19]. Observational studies have also reported significant associations between changes in GIP/GLP-1 and CTX during OGTT [15,16,27]. In the current study, GIP and GLP-1 response during OGTT did not correlate with CTX, but glucose response was closely related to CTX. Results from a study by Nissen and colleagues help shed light on these findings [44]. These authors compared the independent and combined effects of hyperglycemia (vs euglycemia) and GIP infusion (vs saline) on changes in CTX. Hyperglycemia and GIP infusion independently resulted in decreases in CTX, but this effect was more pronounced when GIP infusion was combined with hyperglycemia, suggesting that plasma glucose at least in part influences incretin-mediated bone resorption. In an earlier study from our team [45], increases in GIP correlated with decreases in CTX during OGTT in a sample of young adults with pancreatic insufficient CF, but the majority of participants had either mild glucose dysregulation or diabetes. The participants in the current study were required to have a normal BMI and to be absent of any chronic health conditions known to influence glucose regulation or bone metabolism. On average, fasting glucose was 81 mg/dL and 2-hour glucose was 93 mg/dL, which are indicative of normal glucose control based on American Diabetes Association criteria. Additionally, fasting CTX and BSAP were within normal ranges. For example, average CTX was about 500 pg/mL and the reference range is from 87 to 1200 pg/mL. Thus, we suspect that the null associations between incretin hormones and CTX in the current study is partly attributed to the generally normal metabolic health status of our study sample.

In contrast to the null associations between incretins and CTX, we report significant associations between GLP-1 and both RANKL and BSAP. RANKL is an osteoblast-derived cytokine involved in paracrine regulation of bone metabolism [46], and BSAP is a biomarker of bone formation. RANKL promotes osteoclast differentiation, survival, and function, but OPG acts as a decoy receptor for RANKL to limit bone resorption [47]. BSAP, RANKL, OPG, and the RANKL to OPG ratio were unchanged during OGTT, so interpretation of associations with GLP-1 is unclear. Although the RANKL/RANK/OPG pathway is a pivotal mechanism involved in bone modeling and remodeling [48], involvement of this mechanism in the gut-bone axis requires further attention. This study was not originally powered to observe changes in OPG or RANKL

during OGTT, or associations with incretin hormones, so these preliminary findings require further confirmation.

The well-defined skeletal sexual dimorphism [49,50] underscores the need for studies that identify sex differences during macronutrient ingestion and the incretin and bone metabolism responses that follow. Unfortunately, our study was not sufficiently powered to compare males and females. In adults, *Fuglsang-Nielsen* et al reported that women have higher fasting CTX and P1NP, but that men and women experience similar changes in bone metabolism following OGTT and mixed meal tolerance test (MMTT) [24]. In contrast, in individuals with pancreatic insufficient CF, changes in CTX during OGTT were greater in males vs females. With respect to bone morphology, the differences in bone structure and strength between males and females are substantial. Males tend to have a more robust trabecular bone network and larger cortex compared to females [51,52], but cortical bone density tends to be greater in females vs males [53]. Since 20 % of our study sample was male, we performed sensitivity analyses excluding male subjects to minimize potential confounding of sex. Overall, associations between incretins and bone metabolism during OGTT remained significant after excluding the male subjects. In our total sample, GLP-1 correlated positively with tibia Ct.vBMD, and this association was also maintained in sensitivity analyses including female subjects only. GLP-1 was also marginally associated with lower metrics of cortical bone porosity. Interpretation of these associations are complicated due to our cross-sectional design. However, findings from others suggest that some incretin-based pharmacotherapies, including GLP-1 receptor agonists, have favorable effects on fracture risk and BMD [54–56]. Overall, the results of these sensitivity analyses suggest that our main findings were likely not attributed to sex confounding. Since distinct differences in bone biology [52], as well as metabolic response to food intake exist between males and females [57,58], there is a need to understand sex-related differences with respect to gut-bone cross-talk.

Strengths and limitations

A main strength of this study was our focus on adolescents and young adults around the age of peak bone mass attainment. To this point, all prior studies involving effects of macronutrient/food ingestion on bone metabolism have exclusively included adults [13–17,24–27,37–39]. These prior studies mainly focused on CTX as a biomarker of bone resorption, but we also included alternate biomarkers and bone-derived factors involved in bone turnover. For example, this is the first study to assess RANKL and OPG during OGTT and in relation to incretin hormones. Our results highlight the potential involvement of the RANKL/RANK/OPG pathway in the gut-bone axis, but these preliminary findings warrant additional investigation. Further, assessment of cortical and trabecular bone micro-structure and volumetric density via HR-pQCT addresses critical needs that were described in two separate reports involving determinants of peak bone mass [23,59]. The cross-sectional associations between GLP-1 response and Ct.vBMD reported in this study should be more thoroughly studied prospectively during adolescence and young adulthood. Studies using high resolution bone imaging modalities during the adolescent-to-adult transition are warranted to help understand the underpinning biological mechanisms and contributors to peak bone strength attainment.

The main limitation of this study was our small sample size and cross-sectional design, which limits inference of causality. We were sufficiently powered for our primary aim, which was to test differences in CTX between mins 0 and 120 of OGTT. However, the small sample size likely limited our ability to observe changes in bone formation, or to detect associations between incretins, bone biomarkers, and bone density and morphology. Our sample size also precluded us from testing for interactions between glucose, insulin, and incretin hormones in relation to CTX, and from comparing effects between males and females. Sensitivity analyses excluding the two male subjects yielded similar results to our main findings, indicating that our results are not attributed to sex confounding. We also focused our attention on individuals experiencing the adolescent-to-adult transition due to the lack of studies on this critical life stage, with most prior studies mainly focusing on older adults [60]. Since bone metabolism changes dynamically across the human lifecycle, there is a critical need for studies comparing changes in bone metabolism and incretin hormones following macronutrient ingestion across the spectrum of aging. Alternate experimental methods, such as MMTT, should also be considered in future studies to help facilitate translation to free-living conditions. Finally, additional outcomes such as carboxylated and undercarboxylated forms of osteocalcin might provide unique insights into reciprocal actions in gut-bone cross-talk. Total osteocalcin, which was assessed in this study, is considered a biomarker of bone formation, but the undercarboxylated form of osteocalcin is involved in glucose regulation by augmenting insulin production [61]. Thus, potential bi-directional relationships should be explored.

Conclusions

This study addresses an important knowledge gap involving the role of macronutrient ingestion and gut-derived hormones on bone metabolism during the years surrounding peak bone mass attainment. Since younger individuals have otherwise been excluded from studies involving the gut-bone axis, whether findings from adults are translatable to the transitional years from adolescence to young adulthood is unknown. In the current study, glucose ingestion yielded an acute bone anti-resorptive effect that was consistent with findings from prior studies in adults [13–17,24–27]. Increases in GLP-1, which is an incretin hormone involved in post-prandial insulin secretion [62], was associated with changes in BSAP and RANKL during OGTT, as well as cortical bone density. These results underscore the need for additional research, specifically involving the gut-bone axis, for the acquisition and maintenance of bone mass across the lifespan. Notably, there is a need for adequately powered studies aimed at comparing effects across race, sex, and age groups, as well as dietary or pharmacologic compounds that might help amplify this process.

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CRediT authorship contribution statement

Wang Shin Lei: Project administration, Visualization, Writing – original draft, Investigation, Methodology. **Eugene B.. Rodrick:** Visualization, Writing – original draft. **Staci L. Belcher:** Project administration, Visualization, Writing – original draft, Investigation. **Andrea Kelly:** Visualization, Writing – original draft, Methodology. **Joseph M. Kindler:** Funding acquisition, Project administration, Supervision, Visualization, Writing – original draft, Formal analysis, Methodology, Conceptualization.

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.2023.100314.

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