BONE TURNOVER AND GLP-1 RESPOND TO A PUTATIVE α-GLUCOSIDASE INHIBITOR

By

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ABSTRACT OF THE THESIS

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THESIS Director: Dr. Sue Shapses

The botanical, *Salacia chinensis (SC)*, has α-glucosidase inhibitor (α-GI) properties that attenuates postprandial glycemic indices and increases secretion of glucagon-like peptide (GLP-1), a gut peptide that is associated with a reduced rate of bone resorption. A double-blind, placebo-controlled cross-over study was conducted to evaluate whether *SC* affected bone turnover and could be explained by changes in GLP-1. In this study, 21 healthy overweight/obese adults (body mass index: 29 ± 3.78 kg/m^2; 21-59 years) received either placebo or *SC* with a fixed breakfast at each visit. A fasting blood sample was taken before and at 30-minute intervals after the meal to measure bone turnover markers as well as glycemic indices and gut peptides. Results indicated that *SC* attenuated the bone resorption marker, C-telopeptide of type I collagen (CTX), at 60, 90, and 120 minutes ($p<0.05$), and bone formation marker, osteocalcin (OC), at 180 minutes ($p<0.05$). In addition, *SC* lessened the rise in glucose compared with placebo whereas GLP-1 was increased at 60 minutes ($p<0.05$) with *SC*. Furthermore, GLP-1 and amylin were shown to be predictors of CTX. This study indicates that *SC*, known primarily to minimize the rise in postprandial glycemic indices, also markedly decreases postprandial bone resorption and is associated with a rise in GLP-1. Since *SC* attenuates postprandial bone resorption, longer term use could benefit bone health.

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TABLE OF CONTENTS

LIST OF TABLES

APPENDIX

LIST OF ILLUSTRATIONS

APPENDIX

1. Introduction

Excess body weight is associated with a decline in bone health [1][2]. According to the 2015-2016 National Center for Health Statistics, the prevalence of overweight and obese adults was 31% and 40%, respectively [3]. Similarly, according to the NIH, the prevalence of osteoporosis, a chronic disease characterized by low bone density and reduced bone strength, is significant at approximately 53 million Americans [4]. Osteoporosis develops when bone turnover is unbalanced. Bone turnover is the continuous remodeling of bone beginning with its breakdown through osteoclasts, releasing components of bone tissue composed mainly of type I collagen. Osteoblasts then function to rebuild bone out of these elements. However, when bone resorption significantly outweighs bone formation, there is a resulting decrease in bone quality and strength, increasing the risk of osteoporosis and fractures in overweight and obese adults [5][6].

To measure the rate of bone turnover, certain circulatory or urinary markers can be analyzed [7]. Specifically, for bone resorption, carboxy-terminal telopeptide cross-linked type1 collagen (CTX) and type I collagen amino-terminal telopeptide (NTX) are commonly used [8]. In addition, bone formation can be measured using markers of bone-specific alkaline phosphatase (BALP), procallagen type 1 amino-terminal propeptide (P1NP), procallgen type 1 carboxy-terminal propeptide (P1CP),

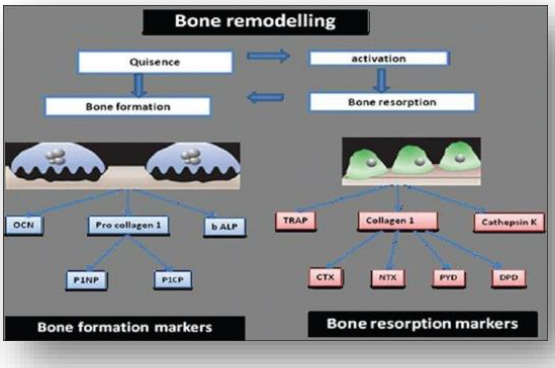


Figure 1. Description of bone turnover [7]

and osteocalcin (OC) [9]. Variability in these markers is common as temporal variations, age, gender, ethnicity, medication, and comorbid conditions, such as, diabetes, can impact the rate of bone turnover [10]. In addition, bone resorption increases at night in comparison to the daytime hours when food is typically consumed. Research has shown that these bone turnover markers are influenced by the ingestion of a meal and the subsequent release of gut hormones, as outlined in the Appendix (Table A1) [11][12],

Certain gut hormones, known as incretins, function to increase the secretion of insulin and effect the rate of bone turnover by binding to its receptors on bone cells [12]. Particularly, after nutrient ingestion, glucagon-like peptide-1 (GLP-1), is secreted from the L-cells primarily in the distal small intestine and the colon in a biphasic pattern [13]. GLP-1 promotes various actions in the pancreas, such as, an increase in β-cell proliferation, insulin secretion and biosynthesis, and a decrease in β-cell apoptosis and glucagon release, ultimately lowering post-prandial glucose levels when GLP-1 is ingested orally rather than through intravenous methods [14]. In addition, GLP-1 increases the release of somatostatin, a peptide hormone that reduces acid secretion and prevents the release of gastrin, secretin, and histamine in the stomach thereby slowing gastric emptying and promoting satiety [13]. Furthermore, research has shown that postprandial GLP-1 secretion is associated with a decrease in bone turnover.

There are various mechanisms by which long-term bone formation is affected by GLP-1. GLP-1 increases the expression of certain genes related to bone formation,

such as Runx2. Runx2 encodes osteoblast-specific transcription factor 2, a transcriptional activator for osteoblast differentiation [15][16]. Additionally, when GLP-1 binds to its receptor, it hydrolyzes glycosylphosphatidylinositol (GPIs), generating inositolphosphoglycans (IPGs), enhancing phosphatidylinositol-3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. These pathways promote cytoprotection, reduce apoptosis of certain tissues, and upregulate OC expression [15][16]. Additionally, GLP-1 also activates the Wnt pathway, promoting osteoblast differentiation from mesenchymal stem cells and maturation, and suppresses sclerostin expression and activity. Sclerostin is a Wnt antagonist that is produced by osteocytes with the purpose of reducing bone formation [17]. The long-term effect of these GLP-1 actions on bone are to increase the rate of bone formation.

GLP-1 affects short-term bone resorption by binding to its receptor on thyroid C cells to promote calcitonin release [6]. In addition, GLP-1 decreases bone resorption over a long period of time through the increase in the OPG (osteoprotegerin) to RANKL (receptor activator of nuclear factor kappa-B ligand) ratio. OPG is known to bind to RANKL to prevent RANKL from binding to its receptor on osteoclasts, inhibiting bone resorption. GLP-1 also increases c-Fos transcription to decrease bone resorption through negative feedback by reducing the bondage of RANKL to RANK [18]. Therefore, promoting the secretion of GLP-1 may improve bone homeostasis.

There have been several other gut hormones and incretins that have been shown to be associated with bone turnover. Similarly to GLP-1, Glucagon-like

peptide-2 (GLP-2), secreted from L cells after the ingestion of food, binds to its receptor on osteoclasts to decrease bone resorption, however the mechanism is still unknown [12]. In addition, glucose-dependent insulinotropic polypeptide (GIP) is secreted from the K cells of the small intestine in response to food consumption [19]. Studies have shown that GIP binds to its receptors found on osteoblasts to stimulate osteoblast differentiation and proliferation and promotes activity through increased expression of type 1 collagen and alkaline phosphatase. However, the method in which GIP reduces osteoclast activity remains controversial [19]. Furthermore, ghrelin, is secreted by endocrine cells in the gastrointestinal tract. When ghrelin binds to its receptor, growth hormone secretagogue (GHS-R), growth hormone is released stimulating IGF-1 secretion from the liver. When IGF-1 binds to its receptor on mature osteoblasts, it signals the PI3K signaling pathway promoting osteoblast activity [20]. Therefore, aside from GLP-1, there are other gut peptides that have been associated with a decrease in bone turnover.

Additionally, glycemic indices have shown to influence the rate of bone turnover as well. Specifically, insulin increases bone formation by binding directly to its receptor on osteoblasts to increase proliferation, function, and maturation [21]. Indirectly, insulin effects parathyroid hormone, IGF-1, and vitamin D which all play a role in bone turnover [21]. Co-secreted with insulin, amylin, a peptide formed in the β-cells of the pancreas, has been found to reduce osteoclast proliferation, however research is limited [22]. In regard to bone formation, amylin increases cAMP to activate the MAPK pathway to promote osteoblast proliferation [22]. Therefore, as

seen in the appendix, a mixed meal can determine the acute effects of GLP-1, GLP-2, GIP, insulin, ghrelin, and amylin on bone turnover (Table A1) [6][11][12][23-31].

Several studies have indicated that while GLP-1 levels are similar between obese and lean persons before a meal, postprandial GLP-1 concentration is significantly blunted in overweight/obese individuals [32][33]. Like the overweight/obese population, individuals with type 2 diabetes mellitus (T2DM) have an impaired release of GLP-1 [34]. Specifically, for T2DM, to promote GLP-1 secretion, α-glucosidase inhibitors (α-GIs), such as, acarbose, have been used [34]. Research has shown that using α-GIs with a mixed meal tolerance test, increases GLP-1 secretion and is associated with a decrease in bone turnover [23]. This type of inhibitor impedes α-glucosidase, a hydrolase enzyme found in the brush border of the intestine that breaks down the α-bonds in complex carbohydrates for absorption to ultimately reduce carbohydrate absorption [34]. Hence, the release of GLP-1 in response to a meal is further increased with α-GIs, a treatment used in diabetes, and may affect obese and lean individuals differently [23].

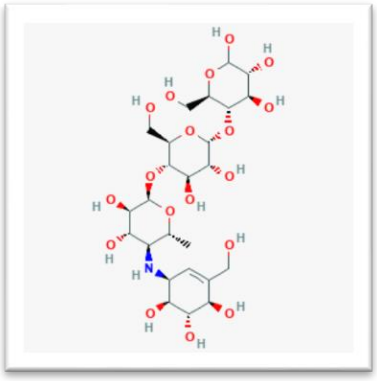


Figure 2. The structure of acarbose [33]

Salacia chinensis (SC) has similar actions to acarbose, acting as a putative α-GI. *SC* is known as a shrub native to India and Sri Lanka that has been used in the treatment for asthma, rheumatism, gonorrhea, skin diseases, and most importantly its suppression of postprandial hyperglycemia [35][36]. *SC* contains medicinal properties found in the leaves, stem, and bark due to several sulfonium sulfate compounds that

have been isolated, such as, salacinol, kotalanol, salaprinol, and mangiferin [35] [37] [38][39]. Of these compounds, mangiferin is the most potent α-GI [40].

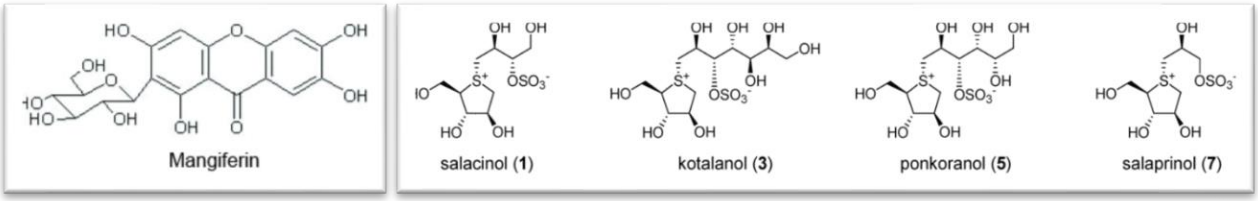


Figure 3. Several compounds extracted from *Salacia chinensis* [38][39].

Other studies have shown that these components found in *SC* demonstrate a higher inhibitory effect on α-glucosidase compared with acarbose [38]. In addition, several researchers reported that after the ingestion of *SC* at one dose and long-term (12 weeks), there were no adverse effects that were typically seen with α-GI medications [41-43]. Therefore, a primary study in this laboratory was conducted with an aim to determine *SC* effects on hunger, glycemic indices, gut hormones, and taste sensations in healthy overweight/obese persons after a mixed meal [44]. As expected, *SC* reduced postprandial glycemic indices with an effect on gut peptides, and there were no reported adverse effects. In addition, a meal with *SC*, hunger was attenuated in the females among the group, but there was no effect on the other markers of appetite [44]. However, there is no study on *SC* and its effect on bone turnover after a mixed meal tolerance test.

This secondary analysis addresses the following aims in overweight and obese individuals:

1. To determine the acute effects of a mixed meal and *SC* on postprandial bone turnover.

2. To examine whether postprandial incretins and hormones predict changes in bone turnover.

It is hypothesized that a putative α-glucosidase inhibitor, SC, will attenuate the postprandial response of bone resorption compared with placebo in overweight and obese subjects. In addition, it is hypothesized that postprandial GLP1 will exhibit a greater rise after a putative α-glucosidase inhibitor compared with placebo and will explain the variance in postprandial bone resorption.

2 Methods

2.1 Participants

Patients were recruited at the Department of Nutritional Sciences at Rutgers University in New Brunswick, NJ through the newspaper, listserves, and Facebook advertising. Preliminary screening included body mass index, medical history, dietary intake, and a short physical exam to ensure absence of disease. Eligible patients included men and women with a BMI 25 to $\leq$ 35 kg/m^2 and between the ages of 21-59 years. Individuals were excluded if they had a current diagnosis of an eating disorder, gastrointestinal illness, bariatric surgery, hyperparathyroidism, untreated thyroid disease, diabetes, blood pressure over 140/90, significant immune, hepatic, or renal disease, significant cardiac disease, active malignancy, or cancer therapy within the past year, and/or current use of obesity medications. Three individuals were excluded from the study as outliers (Figure 4). A written informed consent approved by Rutgers University Institutional Review Board was obtained from all the subjects.

2.2 Study Design and Procedure

This study was a randomized, double-blind, placebo-controlled, crossover clinical study conducted over three visits once a month in New Brunswick, NJ [44]. The test days were separated by one month and conducted during the same time period during the menstrual cycle to attenuate differences in estrogen levels between visits. Based on previous research, six to nineteen participants would allow us to detect a 7% difference in postprandial bone turnover markers between the placebo and *SC* groups, in healthy and obese individuals (80% power and $p < 0.05$) [45][46]. In the original study, the patients were instructed to consume a similar dinner before each of the visits and to arrive at the facility fasting (no food intake after 9 pm) [44]. At each visit, the patients were provided a mixed meal with either control, or one of two doses of *SC* (300 or 500 mg) to consume within 10 minutes (Table 1). Prior to conducting the study, battery toxicology studies were conducted on the *SC* pills and were approved by the FDA and GRAS to be used in food and beverages. The capsules containing *SC* were color and size matched to the placebo pills (OmniActive Health Tehcnologies Ltd., Morristown, NJ, USA). After thirty minutes, a satiety survey was filled out by the participants for the primary objective of the study and their blood was drawn for a total of three hours [44]. In this study, the higher dose of 500 mg of *SC* in comparison with placebo was used because there were less consistent effects of *SC* at the lower dose in the original trial on appetite [44].

Table 1. Composition of mixed meal

Breakfast	Calories Kcal	Carbs g	Fat g	Protein g	Calcium mg	Vitamin D IU
Orange juice – Raw (4 fl oz.)	56	13	0	1	14	69
Skim – Milk (0.5 cup)	42	6	0	4	150	58
Imperial – Margarine (0.5 Tbsp/14g)	35	0	4	0	0.4	30
Egg – Medium Hard-Boiled Egg	60	0	4	6	25	41
Mayers – Italian White Bread (1 slice)	80	16	1	3	40	0
Mixed Meal (% of energy/RDA)	273	35 (50%)	9 (30%)	14 (20%)	230 (23% of RDA)	19 (33% of RDA)

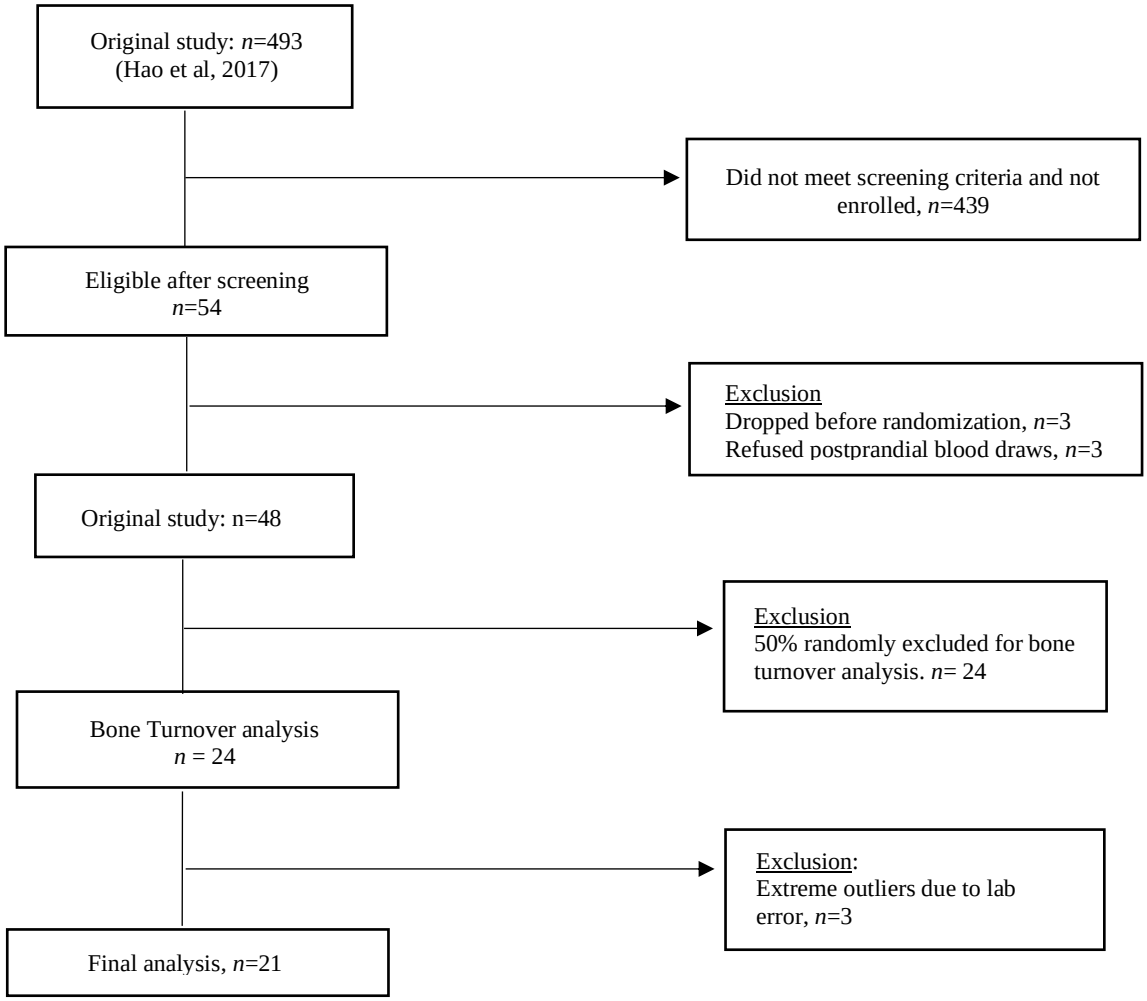


Figure 4. Consort diagram of study participants. At each intervention, in a randomized double-blind cross-over design, subjects were randomized to either placebo or one of the two different doses of *SC***.** Sub-analysis was conducted in the current study on a sub-set of the participants.

2.3 Measurements

2.3.1 Serum Biochemistry

Over the three study visits, six blood samples were drawn in serum-separating tubes and left at room temperature before centrifugation (1000 X g) at 4°C for fifteen minutes. The serum was then aliquoted into labeled microcentrifuge tubes that contained

a protease inhibitor. These were then placed in a sample box and placed in the -80℃ freezer in order to maintain integrity of the sample. Bone resorption was measured using CTX, which is released into circulation after osteoclasts resorb the carboxy-terminal end of type 1 collagen found in bone. CTX is considered the marker of choice compared with the other bone markers when determining the rate of bone resorption [47]. CTX was measured using a human CTX Enzyme Linked Immunosorbent Assay (ELISA; MyBiosource, CA; CV<10%). CTX bone marker range varies in different age groups. The normal range for 18-30 years is 155-873 pg/ml, 30-50 years is 93-630 pg/ml, and from 51-70 years is 35-836 pg/ml [48]. Bone formation was measured using OC, which is a small protein found in bone. Studies have shown that OC is commonly used due to its tissue specificity with low variability [9]. OC was measured with a commercial ELISA Kit (Genway Biotech Inc., CA; intra-assay CV (3.0-4.6%) and inter-assay CV(3.5-5.5%). The normal range for OC for 18 years and older is between 9-42 ng/ml [48]. Glucose, insulin, GLP-1, amylin, and ghrelin were measured in the original research paper [44].

2.3.2 Statistical Analysis

Descriptive statistics of glycemic indices, gut peptides, and bone turnover markers were calculated. Logarithmic transformations were utilized to transform skewed data to normality. Generalized linear models for repeated measures ANOVA was performed to determine within and between-group comparisons of means from before and after the mixed meal at each postprandial time point (0, 30, 60, 90, 120, and 180 min). Additionally, individual values were adjusted for baseline if there were differences between treatment days. The area under the curve (AUC) and integrated AUC (iAUC) for

peak areas of variables were calculated as seen in the Appendix (Figures A2-A3). The variability in response to sex (male vs. female), age (< or > 33 years), and BMI (overweight vs. obese) on outcomes was also examined. One-way ANOVA was used for between-group comparisons. We also compared *SC* vs. placebo at each time point by Student's t-test. Pearson correlations were used to determine relationships between glycemic indices and gut peptides with bone turnover markers. Furthermore, the peak areas of glycemic indices, gut peptides, and bone turnover markers for AUC and iAUC were calculated to analyze the relative influence of independent variables (GLP-1, insulin, ghrelin, and amylin) on CTX and OC using multiple linear regression analysis. A p value of <0.05 was considered statistically significant. Data are represented as mean ± SD, unless otherwise indicated. All statistical analyses were performed using SPSS statistical software (IBM, version 24.0).

3 Results

3.1 Baseline Characteristics

Table 2 provides baseline characteristics for subjects who participated in this study. Sixty-seven percent of the participants were women (n=14) and Caucasian (n=12), while the remaining volunteers were South Asian (n=4), African American (n=2), Asian (n=2), and Hispanic (n=1) who ranged between 21-59 years of age. Weight varied between overweight to stage two obesity (29 ± 3.78 kg/m^2). Sex and BMI were analyzed as covariates but did not significantly alter the results.

Table 2. Baseline characteristics of participants	
Age (y)	33±14
Gender	Female (58%)
Height (m)	1.7 ± 0.11
Weight (kg)	84.5 ± 18.0
Body Mass Index (kg/m^2)	29.0 ± 3.8
Body Fat (%)	27.0 ± 7.3
Waist Circumference (cm)	98.0 ± 17.1
Glucose (mg/dL)	93.4 ± 9.6
Insulin (U/L)	15.7 ± 6.21
GLP-1 (pg/mL)	82.4 ± 30.5
Ghrelin (pg/mL)	408.3 ± 265.1
Amylin (pg/mL)	19.0 ± 10.8
Parathyroid Hormone (pg/mL)	52.8 ± 14.31
25-hydroxyvitamin D (ng/mL)	24.4 ± 8.20
Values are mean ± SD (*n*=21)	

3.2 Serum Bone Turnover Markers

After consumption of a mixed meal, repeated measures ANOVA indicated there was a treatment by time effect for serum CTX (Figure 5A, p=0.013) and serum OC (Figure 5B, p=0.04). Specifically, serum CTX values were lower for *SC* compared with the placebo group at 60, 90, and 120 minutes (Figure 5A, p<0.01). In addition, serum OC tended to be lower with *SC* than placebo but only became significant at 180 minutes (Figure 5B, p=0.001). The iAUC was significantly different between groups for serum OC (Appendix, Figure A2, p=0.001), but not serum CTX. Outlined in the appendix, we also examined the serum CTX response in younger (<33 years) and older (>33 years)

subjects. The repeated measures ANOVA was only significant for the older group (p=0.039) and showed a trend for the younger individuals (Figure A4; p=0.094).

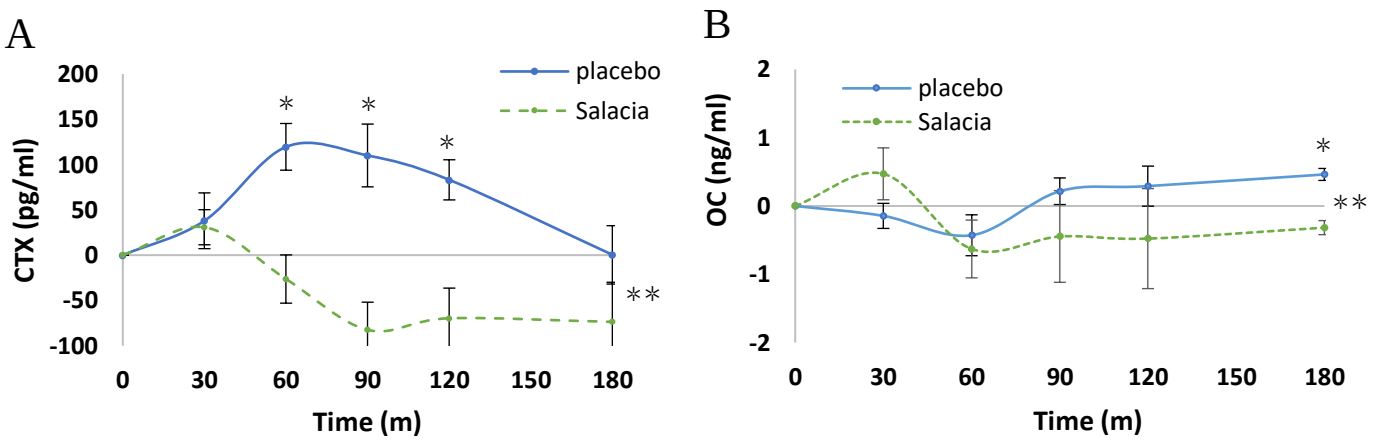


Figure 5. Mean values (± SEM) for serum (A) CTX (B) OC adjusted for baseline before and after a mixed meal with either placebo or *SC* (*n*=21). ** p < 0.01, Differs between groups (repeated measures ANOVA). * p≤0.05, CTX differs between groups at 60, 90, and 120 min and for OC at 180 min.

3.3 Serum Glycemic Indices and Gut Peptides

Repeated measures ANOVA indicated that over time, after the mixed meal, there was a significant treatment effect for serum insulin (Figure 6B, p<0.05), and a trend for serum glucose (Figure 6A, p< 0.1) with *SC* compared with the placebo group. There was an attenuated rise in serum glucose with *SC* compared with the placebo group after a mixed meal at 30 minutes (Figure 6A, p=0.075). Also, postprandial AUC differed between groups for serum glucose (Appendix, Figure A2, p=0.03). Furthermore, the increase in serum insulin was reduced at 30 and 60 minutes but was greater than placebo at 90 minutes (Figure 6B, p<0.01). In addition, after the peak response for serum amylin, the values were lower at 60 minutes in the *SC* group compared with placebo (Figure 6C, p=0.018). The serum GLP-1 response to *SC* peaked at 60 minutes (Figure 6E, p=0.034)

compared with placebo. Postprandial serum ghrelin showed no differences between *SC* and placebo groups (Figure 6D, p>0.10).

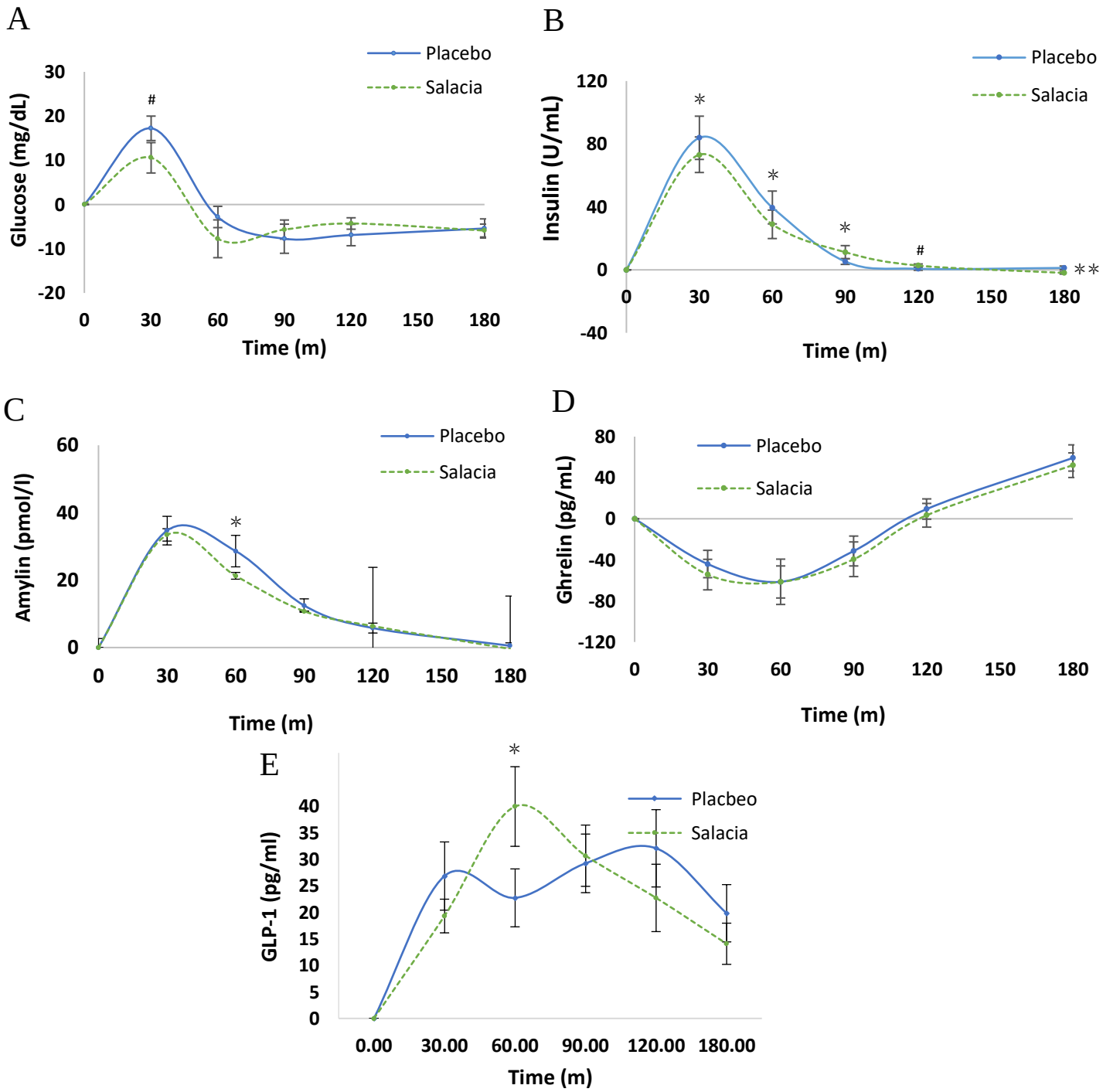


Figure 6. Mean (± SEM) values in serum (A) glucose (B) insulin (C) amylin (D) ghrelin (E) and GLP-1 adjusted for baseline, before and after a mixed meal with either placebo or *SC* (*n*=21) ** $p < 0.01$, Differs between groups (repeated measures ANOVA). * $p \leq 0.05$, glucose differed between groups at 30 min, insulin at 30, 60, 90, and 120 min, amylin at 60 min, and GLP-1 at 60 min.

3.4 Correlations: glycemic indices, gut peptides, and bone turnover markers

Pearson correlations were done separately for placebo and *SC* groups using total and peak iAUC (Appendix, Tables A3 and A4). Specifically, total iAUC indicated that in the placebo group, serum GLP-1 was correlated with insulin (r= -0.78, p=0.001), and was a trend with amylin (r= -0.398, p=0.074) and ghrelin (r= 0.374, p=0.095). In addition, serum amylin was highly associated with insulin (r= 0.568, p=0.007). However, when examining peak iAUC, serum glucose was significantly correlated with amylin (r=0.541, p=0.011), GLP-1 with insulin (r= -0.718, p=0.001), and amylin with insulin (r=0.674, p= 0.001).

In the *SC* group, similar to placebo, the total iAUC for serum GLP-1 was strongly associated with insulin (r= -0.624, p=0.002), and also correlated with ghrelin (r=0.456, p=0.044). In addition, serum glucose was correlated with OC (r= 0.439, p=0.047) and ghrelin with OC (Appendix, Figure A5, r= 0.497, p=0.026). When examining the peak iAUC with *SC* treatment, serum GLP-1 was inversely associated with glucose, amylin, and ghrelin (Appendix, Figure A5), and positively correlated with CTX (Figure 7, $p<0.05$). Additionally, serum ghrelin was positively correlated with OC (Appendix, Figure A5).

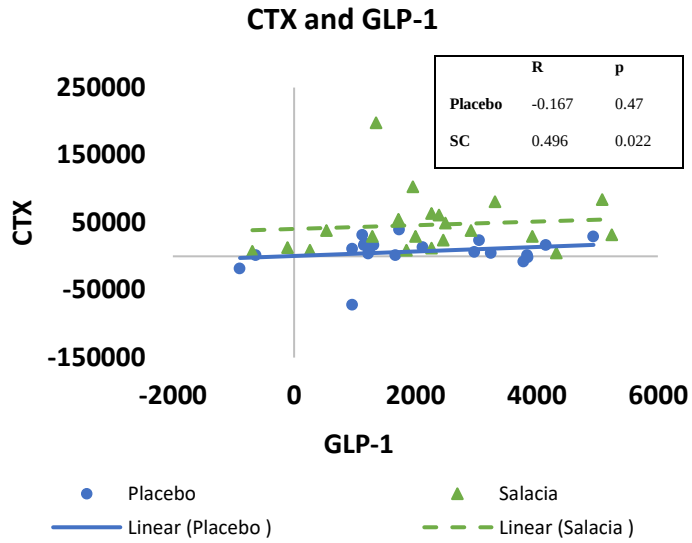


Figure 7. Pearson correlation between glycemic indices and gut peptides with bone turnover markers using peak iAUC values after a mixed meal with *SC* and with placebo (*n*=21). Values are log transformed when non-normally distributed. CTX was directly associated with GLP-1 with *SC*, but not placebo.

3.5 *Predictors of Bone Turnover*

Multiple linear regression analysis was used to determine variables that might explain postprandial changes in bone turnover markers (Table 3). Specifically, serum amylin and GLP-1 have a relationship with serum CTX and as a result may have predicted its outcome in this study. There were no significant predictors for serum OC.

Table 3. Multiple linear stepwise regression analysis for peak AUC CTX

Variable	β coefficient	*P*	Partial R^2	Model R^2
CTX				
Amylin	-0.429	0.003	-0.448	0.270
GLP-1	-0.320	0.025	-0.350	

Abbreviations: CTX C-terminal telopeptide, GLP-1 Glucagon-like peptide-1. *n*=21.

4 Discussion

After the ingestion of a meal, carbohydrates are broken down into digestible carbohydrates through specific enzymes, such as, α-glucosidase. It is likely that the α-glucosidase inhibitor property found in *SC* attenuates the rise of postprandial glucose and insulin secretion, consistent with previous reports examining α-GI medications [49][50]. In this study, a randomized double-blind crossover design was used to determine the effects of the putative α-GI, *SC,* on gut peptides and bone turnover in overweight and obese participants. Serum glycemic indices, gut peptides, and bone turnover markers were measured before and after a mixed meal with either placebo or *SC*. The results indicated that *SC* delayed carbohydrate absorption and decreased the postprandial rise in insulin and amylin (which is co-secreted with insulin from pancreatic β-cells). In this study, we found that a meal did not suppress bone turnover, as found by others outlined in the appendix (Table A5) [45][51][52]. With the addition of a putative α-GI, there was a marked decrease in the rate of bone resorption (CTX-I) with a smaller delayed decrease in the bone formation.

Healthy, lean individuals are characterized by higher levels of serum GLP-1 and other gut peptides than the obese population. Specifically, Verdich et al, showed obese compared with lean individuals had lower serum GLP-1, but after a mean weight loss of 18.8 kg, GLP-1 levels were more like lean subjects [53]. Similar attenuated postprandial GLP-1 results were seen in obese individuals in comparison to age-matched lean individuals and compared with young, lean subjects [54]. This study showed that the overweight/obese population did not have an increase in postprandial GLP-1. However,

the postprandial rise in gut peptides has been reported to increase by α-GI medication taken with a meal in lean and overweight/obese individuals. In a crossover study by Enc et al., a mixed meal tolerance test with acarbose, an α-GI medication, was provided to healthy men and resulted in a significant increase in GLP-1 at 60 minutes [49]. Furthermore, a multi-centered, randomized parallel controlled study determined that when randomly assigning α-GIs, miglitol or voglibose, to fifty overweight, diabetic patients over a 12-week period with a 2-h mixed meal, there is an increase in GLP-1 after 1 hour [50]. This is consistent with findings in the present study, where there was a rise in GLP-1 at 1 hour after the meal with *SC* compared with placebo.

It has been suggested that the elevation in gut peptides after a mixed meal influences bone turnover. Moreover, others have hypothesized whether the amount of calcium, calories, and types or amount of food provided in the mixed meal may affect the response in bone turnover. In particular, Li et al, revealed that food fractionation throughout the day attenuated the postprandial rise in bone resorption, ultimately increasing bone mass in growing rats [55]. Similarly, other studies have shown a similar attenuated rise in postprandial serum CTX in healthy, lean individuals when provided several increasing doses of calcium (250, 500, and 1000 mg), calories (250-3000 kcal), or glucose/fructose/protein [11][56][57].

Consistent with this research, others have examined bone turnover after a mixed meal tolerance test outlined in the appendix (Table A5) [11] [29][45-64]. Most studies agree that there is a decrease in postprandial serum CTX in healthy, lean individuals compared with obese persons, diabetics, and postmenopausal individuals. However, in

reduced obese persons after Roux-en-Y gastric bypass, there was a reduction in postprandial CTX similar to a nonobese persons when given a 250-400 kcal mixed meal [45][51][52]. Conversely, the α-GI medication, vildagliptin, was given with an 890-kcal meal to diabetic individuals, and it had no effect on postprandial CTX compared with the placebo group [58]. In the current study, postprandial CTX did not increase significantly after a mixed meal in the placebo group, but levels were attenuated with *SC*. Therefore, it is possible that the overweight/obese subjects in the current study had some symptoms of preclinical insulin resistance or other comorbid symptoms that may have normalized postprandial bone turnover when a putative α-GI was taken with the meal.

SC treatment in contrast to placebo, caused a significant decrease in bone resorption at 1-2 hours after the meal. Additionally, both serum amylin and GLP-1 were predictors for changes in CTX. Moreover, research indicates that as age increases, serum CTX trends upwards, increasing bone resorption and contributes to age-related bone loss [65]. In this study, regardless of age, *SC* attenuated bone resorption, but *SC* had a more pronounced effect to attenuate postprandial CTX in older compared with younger participants. Also, postprandial OC did not change over time except there was a slight, but significant decrease at 3 hours due to *SC* as compared with placebo treatment. This may reflect coupling of bone turnover with a delayed formation response to the earlier attenuation in bone resorption. This putative α–GI response normalized the postprandial bone turnover response to a meal where bone turnover is typically suppressed [11]. Hence, it is possible that asymptomatic or preclinical metabolic syndrome in the

overweight and obese population alters postprandial bone turnover but can be normalized with an α−GI.

Overall, there are a few novel findings in this study. Unlike previous studies in healthy persons, we found no decrease in bone turnover after a meal and we attribute this to the study design including only individuals who were overweight or obese in this study. It is possible that this abnormal bone turnover response is related to an altered energy metabolism and gut peptide regulation in obese and overweight persons [46][66]. Also, we show that when carbohydrate absorption is slowed, the decreased bone resorption response is more typical to healthy, lean persons. Importantly, we found that GLP-1 may be regulating the bone turnover response with *SC* which acts like other α-GI medications.

5. Strengths and Limitations

A strength of this study is that it is a randomized double-blind crossover design which avoids confounding variables that can occur when recruiting a separate group for each treatment. In addition, we studied healthy overweight and obese individuals and to our knowledge postprandial bone turnover after a standardized mixed meal has not been examined previously in this population. Previous studies have not identified the effects of α-GI medications, or a putative α-GI, such as *SC,* on bone turnover after a mixed meal tolerance test. Another limitation is that the size of the sample was relatively small, and this study was not designed to determine an ideal dose of *SC*, which should be addressed in a future study.

6. *Conclusion*

Overall, these findings show a rise in serum GLP-1 concentration and a decrease in bone resorption with the addition of a putative α-GI, *SC*. In addition, there was a relationship between serum GLP-1 and CTX so that the marked postprandial decrease in CTX with *SC* may be at least partially attributed to the rise in GLP-1. To determine *SC* as an established α-GI, future *SC* studies can be conducted such as, using a 3-arm study with a placebo and a positive control (acarbose) in human or murine subjects. In addition, *in vitro* studies, such as, testing *SC* with positive and negative controls in a petri dish could determine the extent of α-GI activity of *SC*. Furthermore, because a slower carbohydrate absorption using different carbohydrate sources increases bone mineral density and Ca retention in previous studies, using either resistant starch in a murine study or corn fiber in humans, can determine the long-term effects of *SC* or an α-GI on bone [67][68]. This study was not designed to examine other post-prandial measurements of other gut hormones, the long-term effects of multiple meals, or circadian variability in bone turnover. Thus, future studies examining the specific actions of other gut peptides would be of interest. In addition, determining if these findings of bone turnover have clinical significance would be important, particularly in persons with diabetes and those at risk of osteoporosis.

7. Appendix: Table of Contents

Table A1. The effect of incretins on Bone Turnover

Incretin	Secretion	Receptor	Function	Effect	Evidence
GLP-1	Released from the L cells in the small and large intestine in response to feeding	Thyroid C Cell GLP-1 Receptors	Stimulate pancreatic insulin secretion and slow gastric emptying Stimulates secretion of Calcitonin	Decrease in bone resorption	Strong Yamada, 2008, Marathe, 2011, Lu N, 2015, Iepsen, 2015, Henriksen, 2003, Fukase, 1992, Crespel, 1996, Clowes, 2005, Ceccarelli, 2013, Walsh, 2010
GLP-2	Released from the L cells in the small and large intestine after feeding	Receptors are expressed on osteoclasts and in the myenteric ganglia	Enhances mucosal growth and promotion of nutrient absorption. Decreases serum CTX, but no effect on osteoblasts	Decrease in bone resorption	Moderate Lopes, 2015, Holst, 2007, Henriksen, 2003, Clowes, 2005, Walsh, 2010
GIP	Released from the K cells in the duodenum in response to feeding	Receptors are expressed by osteoblasts	Increase osteoblastic number and activity and prevents PTH-induced osteoclast activation	Increased bone formation and decrease bone resorption	Moderate Xie, 2007, Henriksen, 2003, Fukase, 1992, Clowes, 2005, Walsh, 2010
PYY	Secreted from intestinal L cells in the small and large intestine and possibly pancreatic alpha cells in response to feeding	Y receptors are expressed in arcuate nucleus	Signals satiety and reduces nutrient intake	Decreased bone resorption	Moderate Wortley, 2007, Walsh, 2010
Ghrelin	Secreted by the gastric fundus in response to fasting	Growth hormone secretagogue (GHS-R) in the stomach, heart, lung, pancreas, intestine, gonads, adrenal glands, adipose tissue, bone, T cells, pituitary, and hypothalamus.	Stimulate the release of IGF-1 from the liver	Stimulate osteoblast proliferation and differentiation, and inhibits apoptosis	Delhanty PJ, 2014, Walsh 2010, Van Der Velde, 2007, Makovey et al, 2007

Figure A1. Mean values (± SEM) before and after a mixed meal with either placebo or *SC* (*n*=21). **p<0.01, †p<0.10, Differs between groups (repeated measures ANOVA). *p≤0.05, # p<0.10, Differs between groups for a given time point.

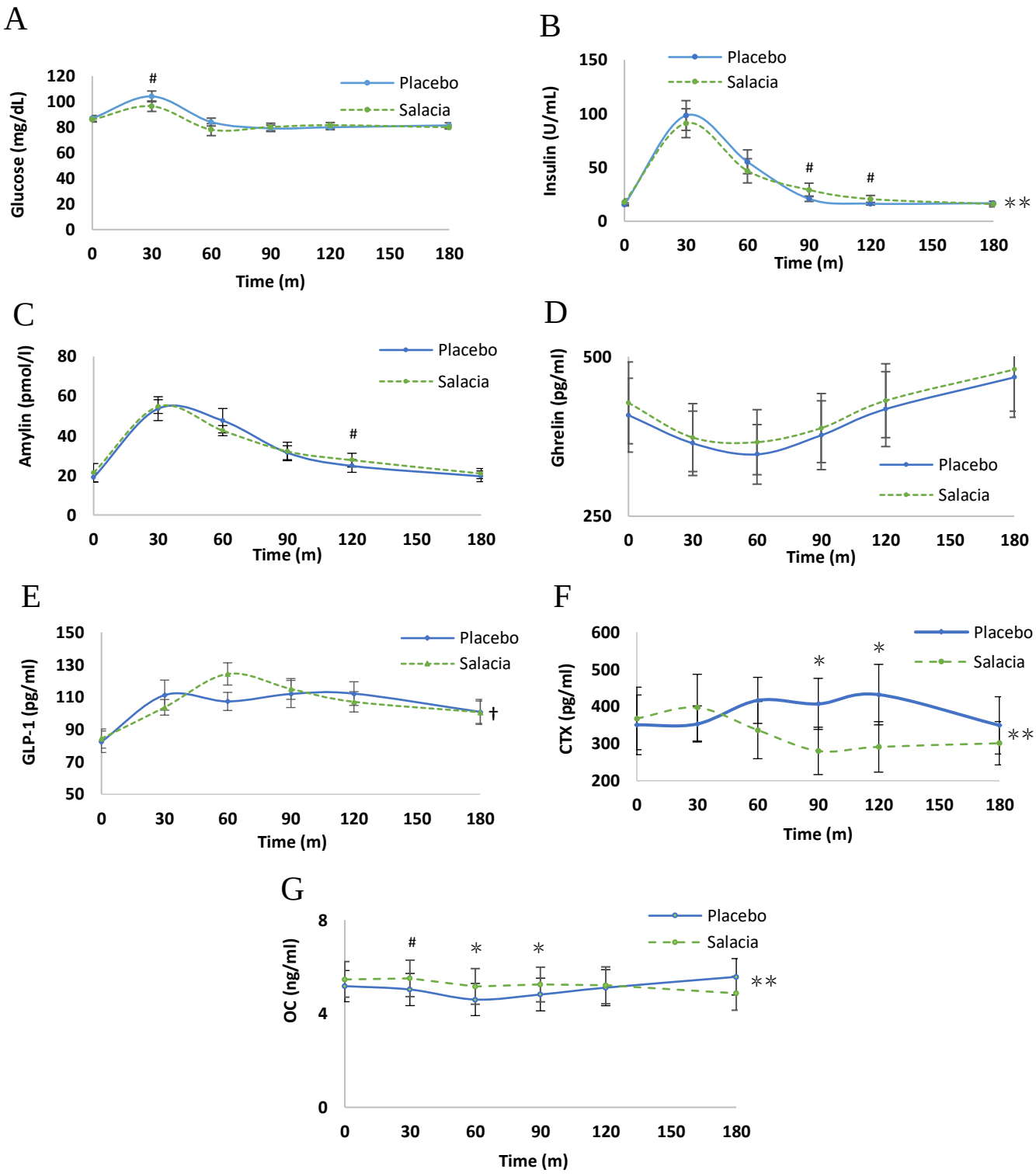


Table A2. Mean Values for glycemic indices, gut peptides, and bone turnover after a mixed meal with placebo or *SC*

	30 minutes	60 minutes	90 minutes	120 minutes	180 minutes
Glucose (mg/dl)					
Control	17.2±12.7	-2.8±11.0	-7.7±15.1	-6.9±11.1	-5.4±9.9
500 mg *SC*	10.6±15.4	-7.8±19.1	-5.7±9.7	-4.3±5.8	-5.9±6.5
Insulin (U/L)					
Control	83.6±60.2	39.6±47.8	5.3±8.4	0.7 ±4.3	1.23±5.5
500 mg *SC*	73.2±51.8	28.93±41.4	11.2±19.2	2.7±4.6	-1.9±3.4
Amylin (pg/ml)					
Control	34.7±20.3	28.6±22.4	12.5±9.5	5.8±7.1	0.5±4.1
500 mg *SC*	33.42±17.7	21.3±15.4	10.8±12.3	6.4±8.4	-0.4±4.6
Ghrelin (pg/ml)					
Control	-44.1±61.0	-61.4±101.2	-31.4±66.7	9.5±45.2	59.2±58.6
500 mg *SC*	-54.4±68.2	-61.6±71.6	-39.43±77.3	69.6±3.4	52.2±12.0
GLP1 (pg/ml)					
Control	26.8±29.4	22.7±24.9	29.2±25.3	32.04±33.4	19.8±24.7
500 mg *SC*	19.3±14.3	39.9±33.5	30.6±25.8	22.7±28.36	16.5±16.3
CTX (pg/ml)					
Control	37.9±144.0	119.6±118.1	110.1±158.9	83.1±101.6	0.3±52.0
500 mg *SC*	30.8±86.6	-26.4±118.9	-82.5±136.7	-69.7±149.1	-73.7±195.6
OC(ng/ml)					
Control	-0.1±0.8	-0.4±0.8	0.2±1.3	0.3±0.9	0.5±0.4
500 mg *SC*	0.5±1.7	-0.6±1.9	-0.5±3.0	-0.5±3.3	-0.3±0.5

Values are represented as means ± SD; *n*=21; *SC*, *SC* extract

Figure A2. Peak AUC (± SEM) before and after a mixed meal with either placebo or *SC* (*n*=21). * p≤0.05, # p<0.1 (one-way ANOVA), Differs between groups for a given time point. The AUC for glucose, insulin, and ghrelin differed with *SC* compared with the placebo group. There were no significant AUC differences due to treatment for amylin, GLP-1, CTX, and OC.

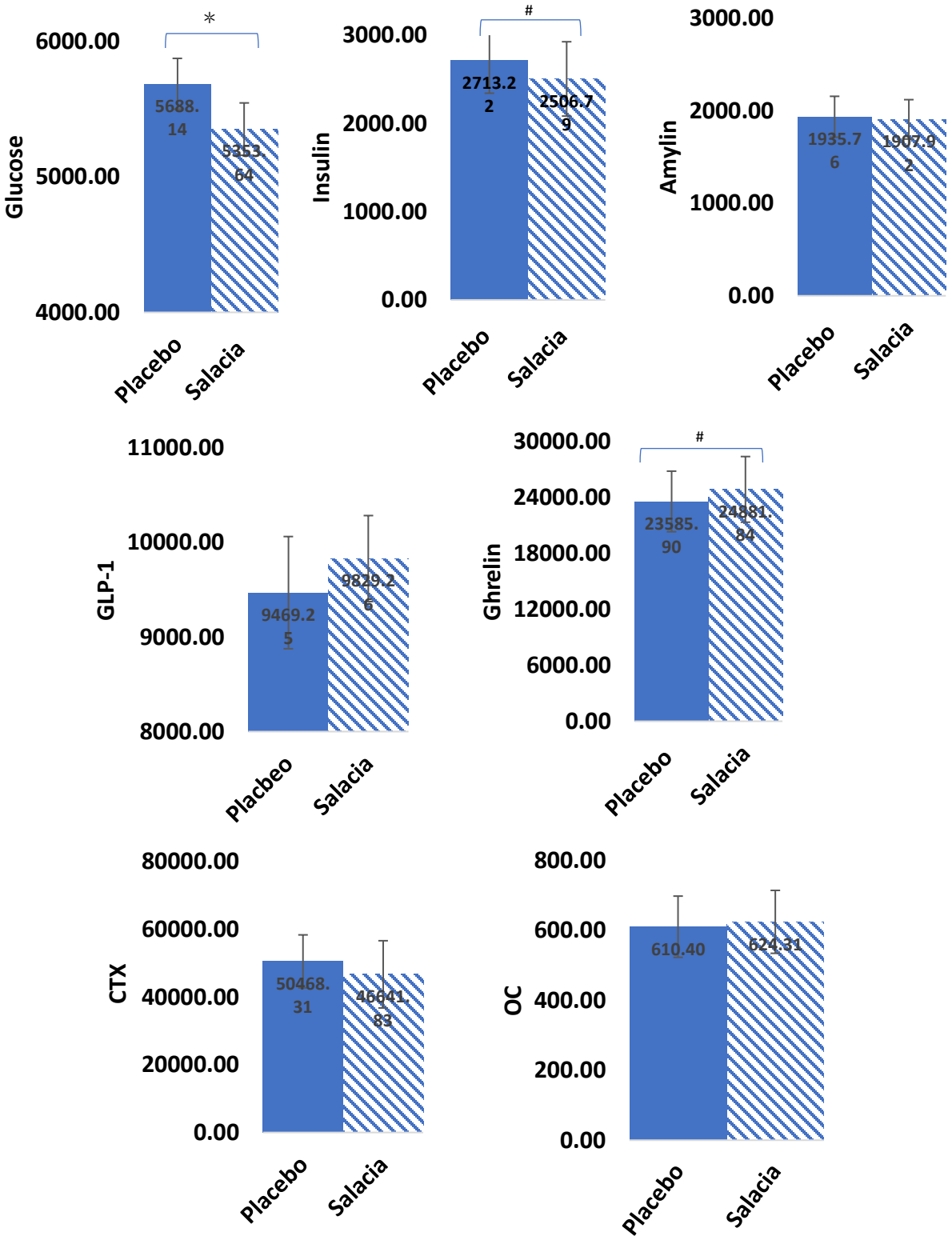
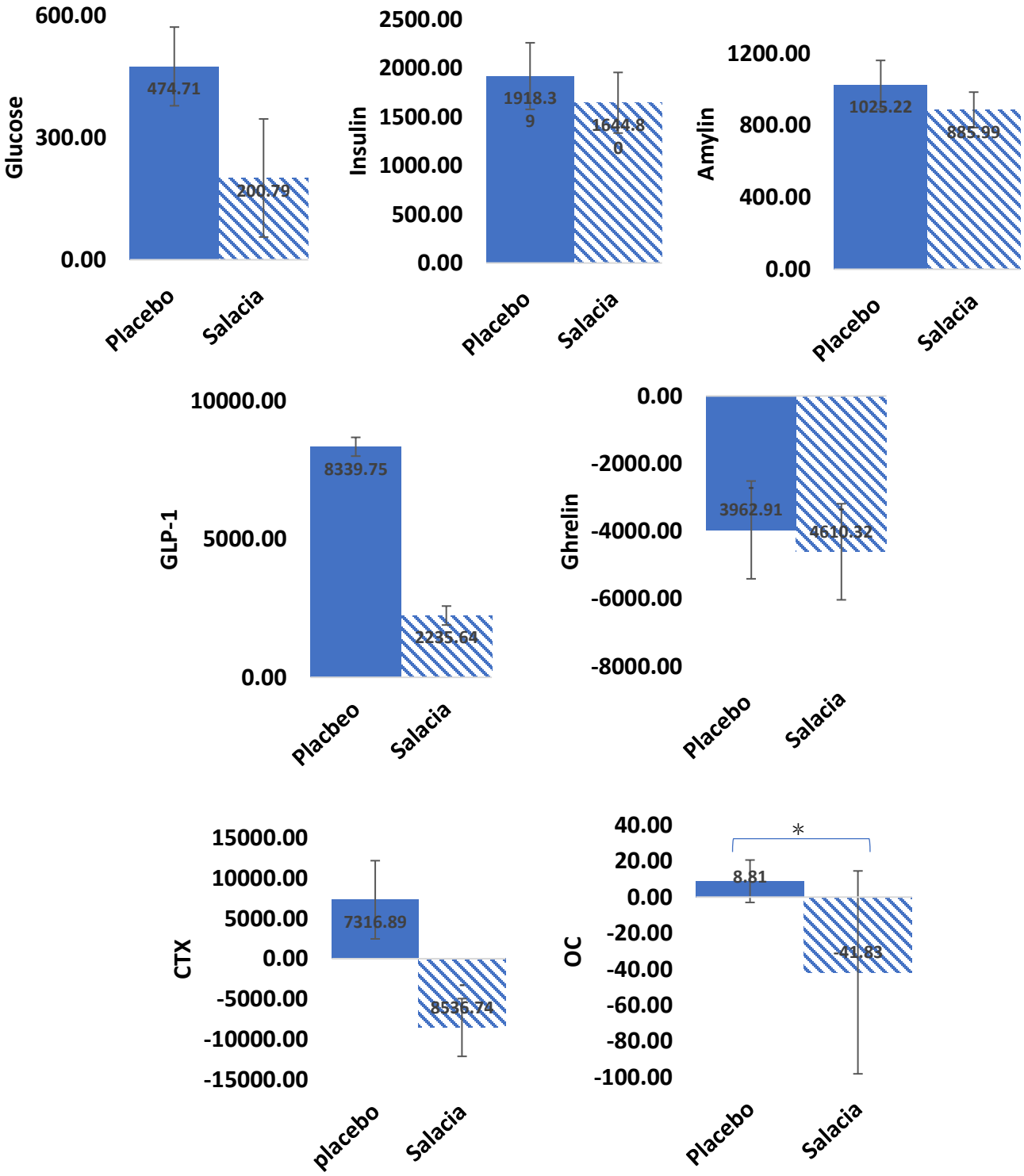


Figure A3. Peak iAUC (± SEM) before and after a mixed meal with either placebo or *SC* (*n*=21). * p≤0.05 (one-way ANOVA), Differs between groups for a given time point. The iAUC for OC differed with *SC* compared with the placebo group. There were no significant iAUC differences due to treatment for glucose, insulin, amylin, GLP-1, and CTX.



Age analysis of postprandial CTX

Objective: To determine whether older compared with younger age differentially influences postprandial CTX.

Hypothesis: The older individuals (>33 years) will have different postprandial CTX response to *SC* compared with the younger population (<33 years).

Method: CTX data was assessed for adjusted or unadjusted values using repeated measures ANOVA on the participants by mean age (< or > 33 years).

Results: Repeated measures ANOVA in the younger population indicated there was an interaction between time and *SC* compared with the placebo group for the adjusted values ($p = 0.094$). However, tests for within-subjects effects using the Greenhouse-Geisser correction showed that the interaction between time and treatment postprandial CTX scores were no longer significant ($f=1.587$; $p=0.217$). For the older group, there was an interaction between time and *SC* compared with the placebo group ($p= 0.077$). Within subject effects showed that there was a significant interaction for postprandial CTX ($f=2.987$, $p=0.039$).

Repeated measures ANOVA in the younger population showed there was an interaction between time and *SC* compared with the placebo group for the unadjusted values ($p=0.017$). However, there was no difference for within subject effects ($f=1.404$; $p= 0.227$). For the older group, there was an interaction between time and *SC* compared with the placebo group ($p=0.021$) and within subject effects showed a significant interaction for postprandial CTX ($f=3.902$; $p= 0.010$).

Conclusions: Older (vs younger) individuals are having a greater effect to suppress bone resorption with *SC* compared with placebo at some time points after a meal. In the total population, the older individuals may be contributing more to the effect of SC to suppress postprandial bone resorption. It is possible that a putative α-glucosidase inhibitor may be beneficial to suppress bone resorption in the more vulnerable aging population who often have higher bone resorption and risk of osteoporosis.

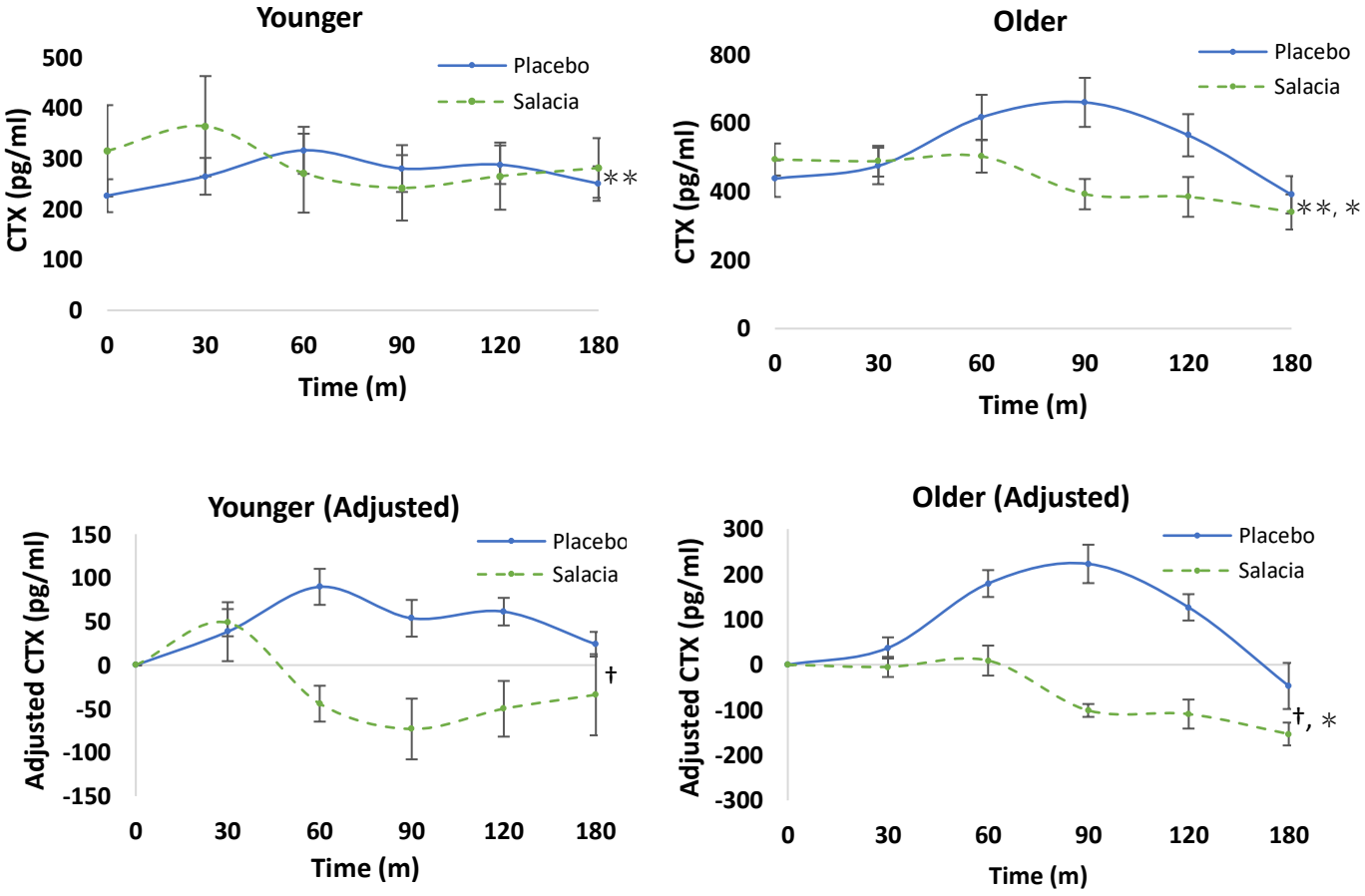


Figure A4. Mean Values (± SEM) of unadjusted and adjusted CTX by age (< and > 33 years) before and after a mixed meal either with placebo or *SC* (n=21). **p<0.01, †p<0.10, Differs between groups (repeated measures ANOVA), ∗p<0.05, within subject effects.

Table A3. Correlations: glycemic indices, gut peptides, and bone turnover after a mixed meal with placebo using total iAUC and log transformed when non-normally distributed

		Glucose	GLP-1	CTX	OC	Amylin	Ghrelin	Insulin
Glucose	Pearson Correlation		0.003	0.245	0.439*	0.18	0.26	0.179
	Sig. (2-tailed)		0.989	0.284	0.047	0.435	0.268	0.437
GLP-1	Pearson Correlation			0.311	0.153	-0.262	0.456*	-.624**
	Sig. (2-tailed)			0.17	0.508	0.251	0.044	0.002
CTX	Pearson Correlation				-0.15	-0.215	0.008	-0.328
	Sig. (2-tailed)				0.517	0.349	0.974	0.146
OC	Pearson Correlation					0.088	0.497*	0.048
	Sig. (2-tailed)					0.705	0.026	0.004
Amylin	Pearson Correlation						-0.248	0.600**
	Sig. (2-tailed)						0.293	0.004
Ghrelin	Pearson Correlation							-0.434
	Sig. (2-tailed)							0.056

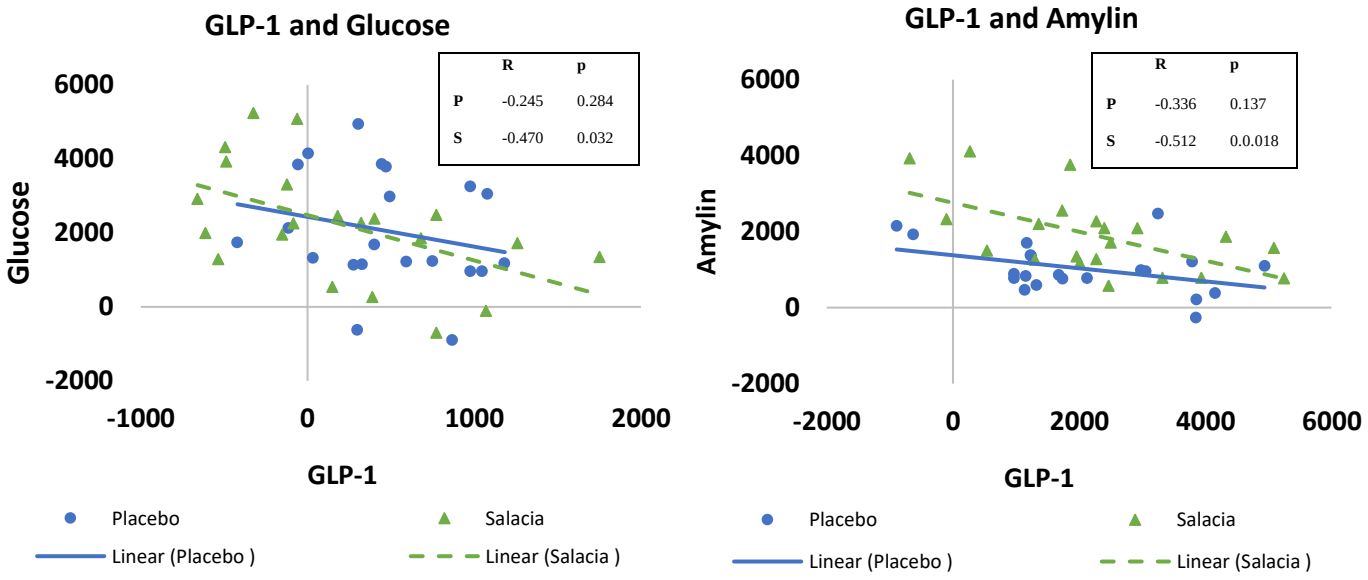
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table A4. Correlations: glycemic indices, gut peptides, and bone turnover after a mixed meal with *SC* using total iAUC and log transformed when non-normally distributed

		Glucose	GLP-1	CTX	OC	Amylin	Ghrelin	Insulin
Glucose	Pearson Correlation		-0.470*	0.007	0.049	0.563**	0.213	0.510*
	Sig. (2-tailed)		0.032	0.976	0.833	0.008	0.353	0.018
GLP-1	Pearson Correlation			0.496*	-0.192	-0.512*	-0.440*	-0.707**
	Sig. (2-tailed)			0.022	0.404	0.018	0.046	0
CTX	Pearson Correlation				0.218	-0.341	0.03	-0.241
	Sig. (2-tailed)				0.343	0.131	0.896	0.293
OC	Pearson Correlation					-0.085	0.585**	0.171
	Sig. (2-tailed)					0.714	0.005	0.459
Amylin	Pearson Correlation						0.186	0.696**
	Sig. (2-tailed)						0.419	0
Ghrelin	Pearson Correlation							0.456*
	Sig. (2-tailed)							0.038

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Figure A5. Pearson correlations between glycemic indices and gut peptides with bone turnover markers peak iAUC values after a mixed meal with *SC* and placebo (*n*=21). Values are log transformed when non-normally distributed. GLP-1 was indirectly associated with glucose and amylin with *SC*, but not with placebo. In addition, OC was directly correlated with ghrelin with *SC*, but not with placebo.



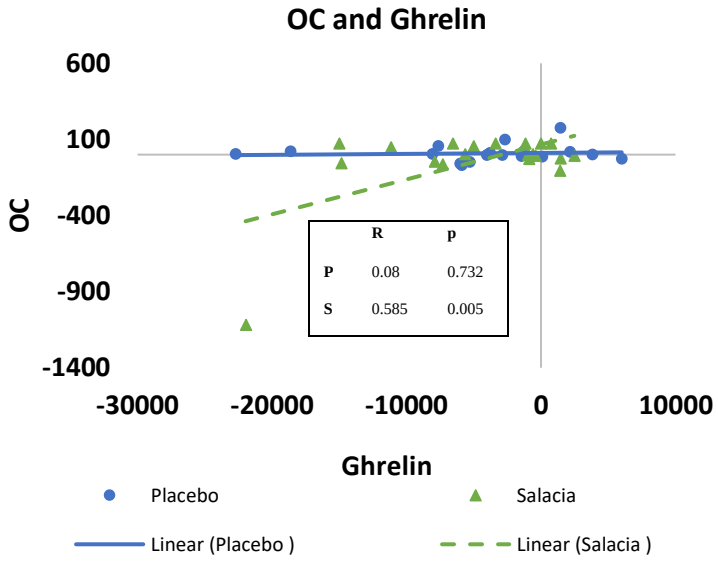


Table A5. Studies of postprandial bone turnover after a meal or glucose load

Author	Age/Population	BMI	Treatment	Meal	Conclusion
Razny U *Nutrients.* 2018	24 Non-Obese 70 Obese (25-65 years)	<30 kg/m^2 30-40 kg/m^2		High fat mixed meal (1027 cal, 30.6 carb, 42.5 g pro, 83.7 g fat)	After HFMTT, OC was significantly reduced after 6 h in non-obese subjects but was suppressed in obese individuals.
Maghsoodi N *Annals of Clinical Biochemistry.* 2016	36 obese indv. (26-64 years)	40.4-46 kg/m^2	Pre and Post Roux-en-Y gastric bypass	400 kcal mixed meal	Prior to surgery, there was no change in postprandial CTX, however post RYGB, there was a significant decrease in postprandial CTX.
Yu E *J Clin Endocrinol Metab.* 2016	19 T2DM individuals (21-65 years)	30-45 kg/m^2	RYGB vs. LAGB	Ensure (40 g carb, 9 g pro, 6 g fat)	Prior to surgery, neither group had a change in postprandial CTX, however a year post surgery, RYGB group had a significant decrease while LAGB remained the same. .
Valderas J *JCEM.* 2014	15 postmenopausal women 15 postmenopausal women (56-66 years)	>35 kg/m^2	RYGB	Standard meal (355 kcal, 50 g carb, 13 g pro, 11 g fat)	Post RYGB, the women had a significant decrease in postprandial CTX in comparison to the controls. P1NP did not significantly decrease.
Kruger M *J Nutr Sci.* 2014	28 Asian Women (20-45 years)	16.7-26.8 kg/m^2	• 250 mg Ca • 500 mg Ca • 1000 mg Ca	200 ml skimmed milk (250 mg Ca)	All three doses of Ca led to a significant decrease in postprandial CTX and PTH. PTH may have influenced the change in CTX.

Ewang-Emukowhate M. A*nnals of Clinical Biochemistry*. 2013	10 healthy men (20-21 years)	21.5-23.8 kg/m^2		400 kcal meal (46.6 g carb, 10.4 g pro, 28.5 g fat)	The decrease in postprandial PTH may influence the resulting suppression of CTX.
Bunck M *Journal of Diabetes.* 2011	59 T2DM (≥ 30 years)	22-45 kg/m^2	Placebo 100 mg of Vildagliptin for 50 weeks	Breakfast – 890 kcals (75 g carb, 35 g pro, 50 g fat)	Regardless of tx, there was a postprandial reduction in CTX.
Yavropoulou M *Journal of Endocrinology. 2011*	118 patients (45 hypothyroid, 40 hyperthyroid, 33 b-thalassemic) 78 healthy individuals (48-53 years)	28-30 kg/m^2		Glucose: 75 g	After oral glucose, CTX (not OC/P1NP) was reduced in all groups specifically in those with hypothyroidism.
Elnenaei M *Ann Clin Biochem.* 2010	20 healthy, lean subjects (27- 30.2 years)	21.3-22.1 kg/m^2	Six meals (250-3000 kcal)	Meals: 500 mg Ca	CTX decreased postprandially. Therefore, the change in CTX was not proportional to calorie contents when calcium is 500 mg.
Henriksen D *JBMR.* 2009	10 healthy individuals (30-40 years)	20.6-24.8 kg/m^2	• Placebo • Glucose • Fat • Protein	Glucose: 75 g (300 kcal) Fat: 70 ml (630 kcal) Pro: 40 g (160 kcal)	Compared with CTX at baseline (21%), there was a significant reduction in CTX with glucose (52%), fat (39%), and pro (52%).
Gottschalck I *Scandinavian Journal of Gastroenterology.* 2008	15 Controls 13 Colectomized with ileostomy 12 Colectomized with jejunostomy (30-65 years)	21.5-24 kg/m^2 19.5-28.3 kg/m^2	• GLP-2 injection • Placebo • Breakfast meal	Breakfast meal 936 kcals – 10% pro, 37% fat, 53% carb.	Patients with a resection of the terminal ileum and colon have postprandial reduction in CTX compared with patients with intact terminal ileum.

		18.1-23.1 kg/m^2			
Chailurkit L *Clinical Endocrinology*. 2007	16 postmenopausal women (Exclusion: hx of T2DM, IFG, IGT (50-88 years).	20-31 kg/m^2		75 g oral glucose tolerance test	After oral glucose, there was a significant decrease in CTX.
Holst J *Scandinavian Journal of Gastroenterology*. 2007	7 healthy controls 7 SBS with colon 7 SBS with colectomy	19.9-24.2 kg/m^2 18.1-23.2 kg/m^2 17.1-26.6 kg/m^2		Breakfast Meal (936 kcal-52% carb, 10% pro, 37% fat)	Healthy controls and SBS patients had a 66% and 27% reduction in postprandial CTX with no response in SBS patients without colon.
Clowes J.A. *Bone*. 2002	20 healthy premenopausal women (21-45 years)	18.7-35.3 kg/m^2	Fasting Breakfast	Self-Selected	All bone formation and resorption markers were significantly decreased postprandial.
Bjarnason N.H. *Bone*. 2002	10 healthy individuals (30-40 years)	22.7 ± 2.1 kg/m^2	Fat Protein Glucose	Fat: 30 mL Pro: 40 g Glucose: 75 g	CTX decreased postprandial.
Li F *JBMR*. 1999.	Growing rats		1.1 g Ca and 1.2 g of Pi 1.1 g of Ca and 0.2 g of Pi		Small meals throughout the day showed in rats to increase bone mineral content, trabecular BMD, and cortical thickness.

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