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Endocrine Functions of Bone Are Suppressed by Orally Administered Glucose Regardless of Bodyweight

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ABSTRACT

Objective: Bone is a target for diabetic complications, but the impact of metabolic changes on bone metabolism is less understood. Bones participate in energy metabolism by via secreted osteokines. Glucose ingestion reduces bone resorption, but the effects on osteokines remain unknown. Further, it is not known whether the response is altered in obesity. The objective of the study was to compare responses in circulating levels of osteokines to glucose ingestion between participants with normal weight (BMI < 26 kg/m^2, $n = 22$) and obesity (BMI > 28 kg/m^2, $n = 27$) (age = 21–54 yr).

Measurements: C-terminal telopeptide of collagen I (b-CTX-I), total osteocalcin (OC), sclerostin, fibroblast growth-factor 23 (cFGF23), uncarboxylated OC, and lipocalin 2 (LCN2) at baseline and after 2 h oral glucose tolerance test (OGTT).

Results: OGTT resulted in a significant decrease in bone resorption, median decrease in b-CTX-I was 42% ($p < 0.0001$) in both groups. Osteokine levels were modestly but statistically significantly decreased, OC decreased by 11% ($p < 0.0001$) and FGF23 by 14% ($p = 0.007$) in both groups, while uncarboxylated OC decreased by 1.2% ($p < 0.0001$) and sclerostin by 7.8% ($p = 0.032$) only in participants with obesity. Although the differences in responses between the groups were not statistically significant, we observed a tendency for less pronounced effect on b-CTX-I ($p = 0.052$) and a greater effect on OC ($p = 0.051$) in participants with obesity compared to normal weight.

Conclusion: Osteokines respond rapidly to oral glucose regardless of body weight. Orally-administered glucose affects not only bone turnover but can also suppress bone endocrine functions. Furthermore, obesity may influence the effect of oral glucose on circulating levels of bone turnover markers.

1 | Introduction

Bone is a dynamic and adaptive tissue that is under constant remodelling. Osteoblasts produce new organic collagenous matrix and incorporate minerals, such as calcium, to form new bone, while osteoclasts resorb bone and release minerals and proteins from the matrix. As the osteoblasts mature, they gradually become embedded into the bone matrix and differentiate into osteocytes that can sense mechanical loading and maintain bone structure and strength. Bone tissue is known to respond to various physical, hormonal, and metabolic stimuli to maintain its metabolic and structural functions.

Bone cells interact with other tissues via a complex secretory system. Besides the paracrine signalling and local communication

between the bone cells, which is mostly related to the regulation of bone turnover, the bone cells also exert endocrine functions and regulate extra-skeletal tissues through circulating endocrine factors called osteokines [1–3]. Several osteokines secreted by osteoblasts and osteocytes have been identified, including osteocalcin (OC), fibroblast growth factor 23 (FGF23), sclerostin, and lipocalin 2 (LCN2) [1].

Osteocalcin (OC) is the most abundant non-collagenous protein produced by the bone-forming osteoblasts and widely used as a marker of bone formation. OC has three glutamic acid residues, which are gamma-carboxylated when it is bound to bone, and can be partially ($g_{1/2}$OC) or completely (g_0OC) uncarboxylated when OC is secreted or released during resorption [4]. Uncarboxylated OC is known to promote insulin secretion from the pancreas and glucose uptake in the skeletal muscles at least in mice [5, 6]. Mouse studies have also shown that another osteoblast-derived osteokine, LCN2 (also known as neutrophil gelatinase-associated lipocalin or NGAL), regulates appetite via hypothalamus and can indirectly affect body weight [7]. However, LCN2 is also expressed in other cell types, such as neutrophils and mesenchymal stromal cells present in the bone marrow, and its role as an osteokine in humans is not completely understood [8]. Besides osteoblasts, the osteocytes also secrete osteokines, such as sclerostin, which is traditionally known as an inhibitor of Wnt-signalling and bone formation but can also regulate, for example, the expansion of white adipose tissue [8]. Another osteocyte-derived osteokine, FGF23, acts on kidneys to regulate phosphate and vitamin D metabolism [9].

Nutrition is known to play an important role in bone metabolism, adding complexity to the system and its regulation. Variations in nutrient intake can transiently affect bone homoeostasis, as bone resorption is known to be postprandially suppressed within 20 min and then recovered to baseline in about 6 h, when assessed by circulating levels of bone resorption markers [10]. This is supported by our findings [11, 12] and those of others [13–15], reporting up to 60% decrease in circulating levels of bone resorption marker beta C-terminal telopeptide of collagen I (b-CTX-I) after a 2 h standard oral glucose tolerance test (OGTT) [11–14]. Markers of bone formation are also known to be influenced by feeding, but the decrease is less pronounced [10, 15]. Additionally, food intake may, at least partially, contribute to the known circadian variation of bone turnover markers [10, 15, 16]. However, it is not known whether the ingestion of a high glucose dose also affects the circulating levels of osteoblast and osteocyte-derived osteokines.

The effects of orally administered glucose on the levels of circulating osteokines have not been previously studied in humans in the context of normal body weight versus obesity. People with obesity generally have lower bone turnover rate as shown by the suppressed circulating levels of bone turnover markers, e.g., b-CTX-I and total OC [11]. Furthermore, individuals with obesity and type 2 diabetes tend to have more fragile bones and increased fracture risk, even if their bone mineral density is normal or high [17, 18]. As there is growing evidence that altered osteokine levels are associated with the whole-body energy metabolism [1], we hypothesised that the response to orally administered glucose could be different in people with normal body weight and in those with obesity.

The aim of this study was to assess the effect of orally administered glucose on the bone turnover markers, b-CTX-I and total OC, and the circulating levels of four osteokines, $g_{0/1}$OC, FGF23, sclerostin, and LCN2, and to compare the response to OGTT between individuals with normal body weight or obesity.

2 | Materials and Methods

2.1 | Study Subjects

Volunteers with normal body weight or obesity were recruited from two larger clinical studies that were performed at Turku PET Centre (MOTORBAT, NCT05468151; TOTAL, NCT06739473). The studies were conducted according to the Declaration of Helsinki and were approved by the Ethics Committee of the Wellbeing Services County of Southwest Finland. The volunteers were informed before the study, and they all signed an informed consent. Overall, 49 participants (males $n = 11$, females $n = 38$) between 21 and 54 years of age were enroled. The number of participants was estimated based on our previous findings [11] to detect 30% difference in total OC levels in response to OGTT between the groups at 80% power. The inclusion criteria for participants with obesity were waist circumference over 80 cm for women and over 94 cm males in the MOTORBAT study and BMI over 30 kg/m^2 in the TOTAL study. In the current study, these participants were considered as the group with obesity. The inclusion criteria for the participants with normal weight were waist circumference below 80 cm for women and below 94 cm for men in the MOTORBAT study and BMI between 18.5 and 25 kg/m^2 in the TOTAL study, and considered here as the group with normal weight. None of the participants with normal weight had a history of obesity or type 2 diabetes. In brief, the exclusion criteria included the use of nicotine products, narcotics, or excessive alcohol, use of GLP-1 agonists or insulin, history of eating disorders, abnormal thyroid function, active infection or cancer treatment, and severe immune disorders. Out of all participants, 29% (14/49) used regular vitamin D supplementation, and 34% (13/38) of female participants were on hormonal contraceptive medication. Two females were receiving supplementary estradiol.

2.2 | Oral Glucose Tolerance Test and Anthropometry

A standardised 2 h Oral Glucose Tolerance Test (OGTT) was performed using 75 g of liquid glucose. Baseline serum and plasma samples were taken after an overnight fast at the latest by 9:30 a.m. and then again 2 h after glucose ingestion. Peripheral blood samples were drawn from the median cubital vein and underwent a standard centrifugation at room temperature before aliquoting and storing at −85°C until analysis. Plasma glucose and serum insulin concentrations were measured at Turku University Hospital laboratory using a hexokinase assay GLUC3 Glucose HK Gen.3 and an electrochemiluminescence immunoassay Elecsys Insulin (Roche Diagnostics, Mannheim, Germany), respectively.

Anthropometry, including body height, body weight and waist circumference, was collected during the study visit at baseline.

2.3 | Bone Turnover Marker and Osteokine Measurements

Serum total OC and plasma uncarboxylated OC ($g_{0/1}$OC) were measured at 0 and 2 h with in-house ELISA assays, which we have previously described, optimised, and validated to specifically target the specific forms of OC. Briefly, two-site immunoassays were used to detect either the N-terminal mid-segment of OC (total OC) [19] or the fully uncarboxylated or mono-carboxylated (at position Gla-24) form of OC ($g_{0/1}$OC) [20]. The monoclonal antibodies (Mabs) used for total OC were 2H9 and 6F9 [19]. Assay for $g_{0/1}$OC uses 2H9 as capture Mab and recombinant antibody Fab-AP13 together with anti-AP Mab for detection [20]. The Mabs were either biotinylated (2H9) or labelled with Europium chelate (6F9, anti-AP) as previously described [20, 21]. Streptavidin-coated microtiter plates and Europium Fluorescence Intesifier were purchased from Uniogen (Turku, Finland), and time-resolved fluorescence was measured using a Victor X4 Multilabel Microplate reader (Perkin Elmer Life Sciences/Revvity, Turku, Finland). Serum sclerostin, FGF23, and b-CTX-I and plasma LCN2 were measured according to manufacturer's instructions with the following commercial ELISA kits: bioactive sclerostin (BI-20472, Biomedica Medizinprodukte GmbH, Wien, Austria), C-terminal FGF23 (cFGF23, BI-20702, Biomedica Medizinprodukte GmbH, Wien, Austria), Serum CrossLaps (b-CTX-I) ELISA (AC-02F1, Immunodiagnostic Systems Ltd, Boldon, UK) and human NGAL (ie, LCN2, HK330, Hycult Biotech, Uden, The Netherlands), respectively. For every ELISA assay, each sample was assessed in duplicate. Baseline and 2 h samples were analysed in parallel to reduce inter-assay variance. The intra-assay variations in this study were 1.7% for total OC, 5.8% for $g_{0/1}$OC, 5.5% for sclerostin, 23.5% for cFGF23, 14.5% for b-CTX-I, and 14.3% for LCN2.

The nomenclature and terminology used in this paper regarding bone turnover markers and osteokines follows the latest international guidelines [22].

2.4 | Statistical Analysis

The data are given as means with standard deviations or medians with interquartile range (IQR). All data were tested for normality before proceeding with further testing. First, a linear regression model was created with adjustment for age, gender, group, and baseline when appropriate. From the model, studentized residuals were plotted and their normality was assessed by visual inspection and Shapiro–Wilk's test. The data were considered normally distributed if the data was symmetrical and the test value was close to 0.95 with a $p > 0.05$. If the data were not normally distributed, the test was repeated after a Ln-transformation. If Ln-transformation did not successfully fulfil the normality assumption, the data were tested with an equivalent nonparametric test as indicated. Baseline characteristics, glucose, insulin, bone-turnover marker, and osteokine measurements were compared between the groups. Age was tested with one-way ANOVA and gender distribution with Fisher's Exact test. Weight and BMI values were adjusted for age and gender and tested with analysis of covariance (ANCOVA). Glucose, insulin, homoeostatic model assessment of insulin resistance (HOMA-IR), bone-turnover markers, and osteokines levels were adjusted for age, gender and group and tested with ANCOVA. The response of bone-turnover markers and osteokines to OGTT was analysed using Wilcoxon signed-rank test separately for each group. To assess whether obesity affected the response of bone turnover markers and osteokines to OGTT, a linear regression model was created with adjustments for age, gender, baseline marker levels, and group. OC, $g_{0/1}$OC, b-CTX-I, cFGF23 was tested with ANCOVA. Sclerostin was tested with nonparametric Kruskal–Wallis test. In addition, Pearson's correlations for bone-turnover markers and osteokines were tested for the baseline values and for the changes over 2 h OGTT. JMP Pro v17.0.0 -software (JMP Statistical Discovery LLC, Cary, North Carolina, USA) was used for the statistical analysis.

3 | Results

3.1 | Baseline Characteristics

Baseline characteristics of the participants are presented in Table 1. The median age of study participants in both groups was similar ($p = 0.81$). Although female participation was higher, 73% and 81% in groups with normal weight and obesity, respectively, both sexes were represented in similar ratios in both groups ($p = 0.51$). As expected, weight and body mass index (BMI) were statistically significantly lower in the group with normal body weight ($p < 0.0001$) with a median (IQR) BMI of 22.0 kg/m^2 (20.2–24.7), while the BMI was 34.1 kg/m^2 (30.9–39.4) in the group with obesity.

At baseline, fasting glucose and insulin levels were significantly higher in the group with obesity with a median (IQR) of 5.5 mM (5.2–5.8) and 11.0 mU/L (8.0–14.0), respectively, than in the group with normal weight with 5.2 mM (4.8–5.5, $p = 0.014$) and 4.5 mU/L (3.0–6.0, $p < 0.0001$), respectively. The calculated HOMA index of insulin resistance was higher in the participants with obesity at 2.7 (2.0–3.4) than in the participants with normal weight at 1.1 (0.78–1.4, $p = 0.0002$). Additionally, baseline levels of bone turnover markers b-CTX-I ($p = 0.062$) and total OC ($p = 0.072$) tended to be lower in the group with obesity. We also observed a significantly higher ratio of uncarboxylated OC to total OC ($g_{0/1}$OC/OC) in the group with obesity ($p = 0.012$) at baseline. However, no statistically significant differences were observed in the baseline levels of $g_{0/1}$OC ($p = 0.87$), sclerostin ($p = 0.14$) cFGF23 ($p = 0.10$), or LCN2 ($p = 0.14$) between the groups (Table 1).

3.2 | Bone Turnover Markers and Osteokines Are Suppressed by OGTT

At 2 h of OGTT, we observed higher levels of plasma glucose in the group with obesity with a median (IQR) of 7.3 mM (6.0–9.8) when compared to the group with normal weight with glucose

TABLE 1 | Participant characteristics and osteokine measurements at baseline and at 2 h after OGTT in the group with normal weight and in the group with obesity.

	Normal weight	Obesity	*p*
n	22	27	
Age (years)	36 (31–43)	37 (33–42)	0.81
Male/Female	6/16	5/22	0.51
Weight (kg)	63.4 (58.4–78.3)	98.4 (87.0–117.8)	**< 0.0001**
BMI (kg/mm^2)	22.0 (20.2–24.7)	34.1 (30.9–39.4)	**< 0.0001**
		Baseline	
Glucose (mM)	5.2 (4.8–5.5)	5.5 (5.2–5.8)	**0.014**
Insulin (mU/L)	4.5 (3.0–6.0)	11.0 (8.0–14.0)	**< 0.0001**
HOMA-IR	1.1 (0.78–1.4)	2.7 (2.0–3.4)	**0.0002**
b-CTX-I (ng/mL)	0.37 (0.29–0.50)	0.29 (0.22–0.43)	0.062
OC (ng/mL)	12.0 (10.7–15.1)	9.6 (8.4–11.6)	0.072
$g_{0/1}$OC (ng/mL)	2.37 (2.22–2.46)	2.31 (2.21–2.44)	0.87
$g_{0/1}$OC/OC	0.20 (0.15–0.22)	0.23 (0.21–0.29)	**0.012**
Sclerostin (pmol/L)	35.3 (21.6–49.8)	39.7 (32.8–52.9)	0.14
cFGF23 (pmol/L)	0.48 (0.28–0.84)	0.87 (0.56–1.62)	0.10
Lcn2 (ng/mL)	13.9 (10.1–18.5)	13.5 (9.8–17.8)	0.14
		At 2 h of OGTT	
Glucose (mM)	5.5 (5.0–6.9)	7.3 (6.0–9.8)	**0.0014**
Insulin (mU/L)	19.5 (13.8–33.5)	66.0 (48.0–85.0)	**< 0.0001**
b-CTX-I (ng/mL)	0.22 (0.15–0.27)	0.18 (0.12–0.24)	0.60
OC (ng/mL)	10.9 (9.2–13.4)	8.5 (7.0–10.8)	**0.023**
$g_{0/1}$OC (ng/mL)	2.37 (2.2 –2.44)	2.26 (2.12–2.40)	0.54
$g_{0/1}$OC/OC	0.22 (0.18–0.24)	0.27 (0.21–0.31)	**0.0045**
Sclerostin (pmol/L)	35.0 (21.8–47.2)	37.4 (32.2–48.8)	0.44
cFGF23 (pmol/L)	0.50 (0.25–0.77)	0.85 (0.45–1.24)	0.17
Lcn2 (ng/mL)	11.7 (9.4–16.4)	13.1 (8.7–16.2)	0.99

Note: Data are shown as median with interquartile range (in parentheses). Age was tested with one-way ANOVA and gender distribution with Fisher's Exact test. Weight and BMI values were adjusted for age and gender and tested with analysis of covariance (ANCOVA). Glucose, insulin, homoeostatic model assessment of insulin resistance (HOMA-IR), bone-turnover markers, and osteokines levels were adjusted for age, gender and group and tested with ANCOVA. *p* values indicate the difference between groups with normal weight and obesity and statistically significant values ($p < 0.05$) are marked in bold.

levels of 5.5 mM (5.0–6.9, $p = 0.0014$, Table 1). In the group with obesity, also insulin levels were approximately three-fold higher after OGTT (66 mU/L, IQR 48–85) when compared to the group with normal weight (19.5 mU/L, IQR 13.8–33.5, $p < 0.0001$). The OC levels after OGTT were significantly higher in the group with normal weight than in the group with obesity ($p = 0.023$), while the ratio of $g_{0/1}$OC/OC remained higher in the group with obesity ($p = 0.045$, Table 1).

We then compared the changes in bone turnover marker and osteokine levels over the 2 h OGTT. We observed a decrease in b-CTX-I levels in response to OGTT in both groups, with a median (IQR) decrease of 0.17 ng/mL (0.09–0.23, $p < 0.0001$) in the group with normal weight and 0.11 ng/mL (0.07–0.19, $p < 0.0001$) in the group with obesity (Figure 1A). Similarly, circulating levels of total OC were decreased by 1.11 ng/mL (0.69–2.2, $p < 0.0001$) in the participants with normal weight and 1.12 ng/mL (0.91–1.4, $p < 0.0001$) in the participants with obesity (Figure 1B). Uncarboxylated OC and sclerostin were decreased by OGTT only in the group with obesity, and the percentual decreases were modest, 0.030 ng/mL (0.014–0.046, $p < 0.0001$, Figure 1C) for $g_{0/1}$OC and 2.5 pmol/L (−1.9 to 5.8, $p = 0.032$, Figure 1D) for sclerostin. cFGF23 was decreased by 0.071 pmol/L (0.0098–0.16, $p = 0.0073$) in the participants with normal weight and 0.11 pmol/L (−0.039 to 0.32, $p = 0.0039$) in the participants with obesity (Figure 1E). LCN2 did not change in response to OGTT in either group ($p = 0.47$ and $p = 0.44$, Figure 1F).

3.3 | The Acute Osteokine Response to OGTT Is Similar in Normal Weight and Obesity

We then wanted to compare the response to OGTT between the participants with normal weight and obesity. Despite observing an overall decrease in bone turnover markers in response to OGTT, there were no statistically significant differences in how the two

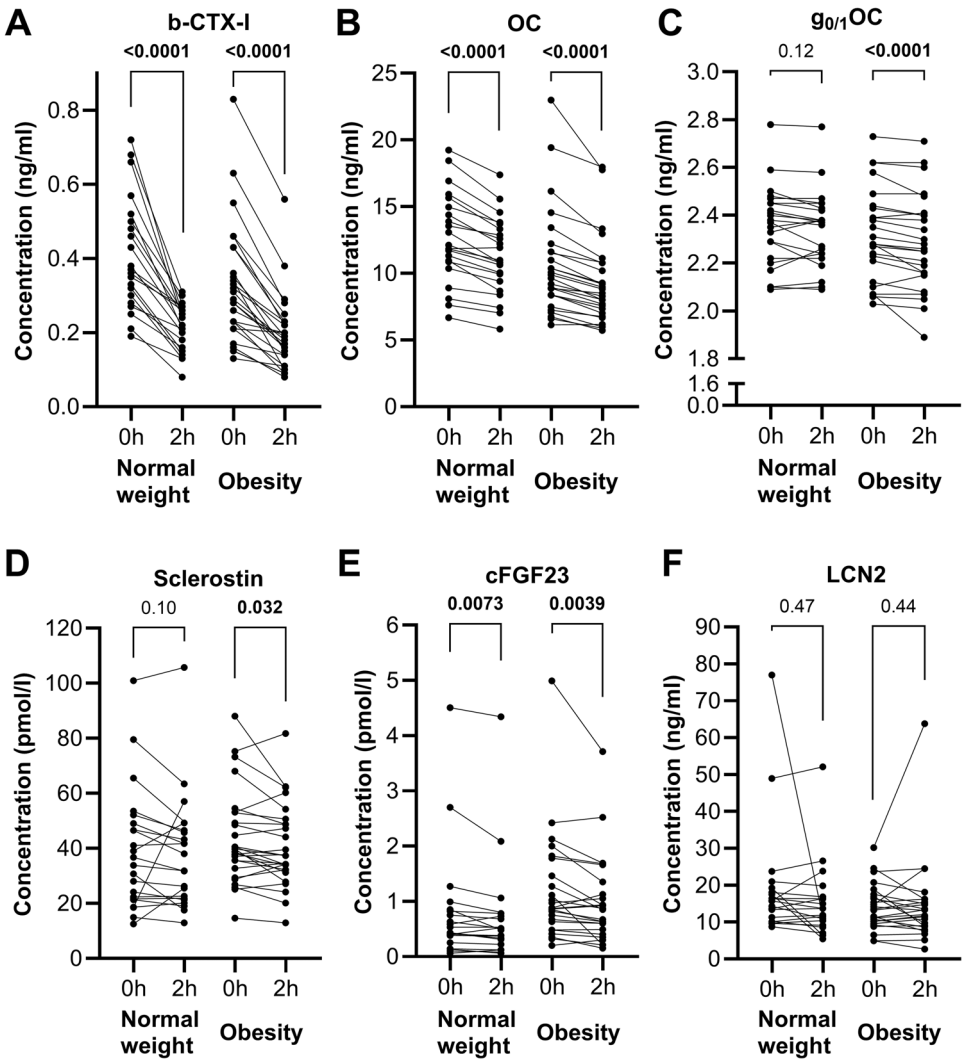

FIGURE 1 | Concentrations of bone turnover markers b-CTX-I and total OC (A, B) and osteokines $g_{0/1}$OC, Sclerostin, cFGF23, and LCN2 (C–F) in participants with normal weight and obesity at baseline (0 h) and at 2 h (2 h) after oral glucose tolerance test (OGTT). Data are shown as individual values at 0 and 2 h with a line connecting paired measurements. The response to OGTT was analysed separately for both groups using Wilcoxon signed-rank test. Statistically significant *p*-values ($p < 0.05$) are marked in bold.

groups responded to OGTT. However, we observed a tendency for a less pronounced decrease in b-CTX-I levels in the group with obesity (i.e., 38%) when compared to the group with normal weight (−46%, $p = 0.052$, Figure 2A). In contrast, we observed a more prominent decrease in OC levels in the group with obesity compared to the group with normal weight, 11% and 9%, respectively ($p = 0.051$, Figure 2B). Similarly, $g_{0/1}$OC tended to decrease more in the participants with obesity (1.3%) in response to OGTT than in the group with normal weight (0.4%, $p = 0.088$, Figure 2C).

No significantly different responses to OGTT were observed in the circulating levels of sclerostin, cFGF23, or LCN2 between the two groups (Figure 2D–F).

3.4 | OGTT-Induced Changes in Osteokines Are Not Associated With Changes in Bone Resorption

Next, we wanted to investigate if the baseline levels of bone turnover markers and osteokines correlate in participants with normal weight or obesity. As expected, total OC positively and strongly correlated with b-CTX-I ($r = 0.61$, $p < 0.0001$; $r = 0.80$, $p < 0.0001$) and $g_{0/1}$OC ($r = 0.76$, $p < 0.0001$; $r = 0.76$, $p < 0.0001$) in participants with normal body weight and with obesity, respectively (Table 2). b-CTX-I also positively correlated with $g_{0/1}$OC ($r = 0.61$, $p = 0.0007$) in participants with obesity, but not in those with normal weight ($p = 0.14$). Baseline levels of cFGF23 did not correlate with bone turnover markers (total OC or b-CTX-I) or other osteokines in either group (Table 2).

Finally, we wanted to analyse if OGTT-induced changes in osteokine levels are associated with changes in bone turnover markers. The changes in b-CTX-I and total OC levels during the 2 h OGTT were positively correlated in both groups ($r = 0.61$, $p = 0.0025$; $r = 0.62$, $p = 0.0006$) (Table 2). Changes in $g_{0/1}$OC and sclerostin ($r = 0.73$, $p = 0.0001$), in total OC and $g_{0/1}$OC ($r = 0.38$, $p = 0.078$), and in total OC and sclerostin ($r = 0.38$, $p = 0.083$) were positively correlated in the group with normal weight but not in the group with obesity (r values from 0.10 to 0.25, $p > 0.05$). However, the changes in sclerostin levels in

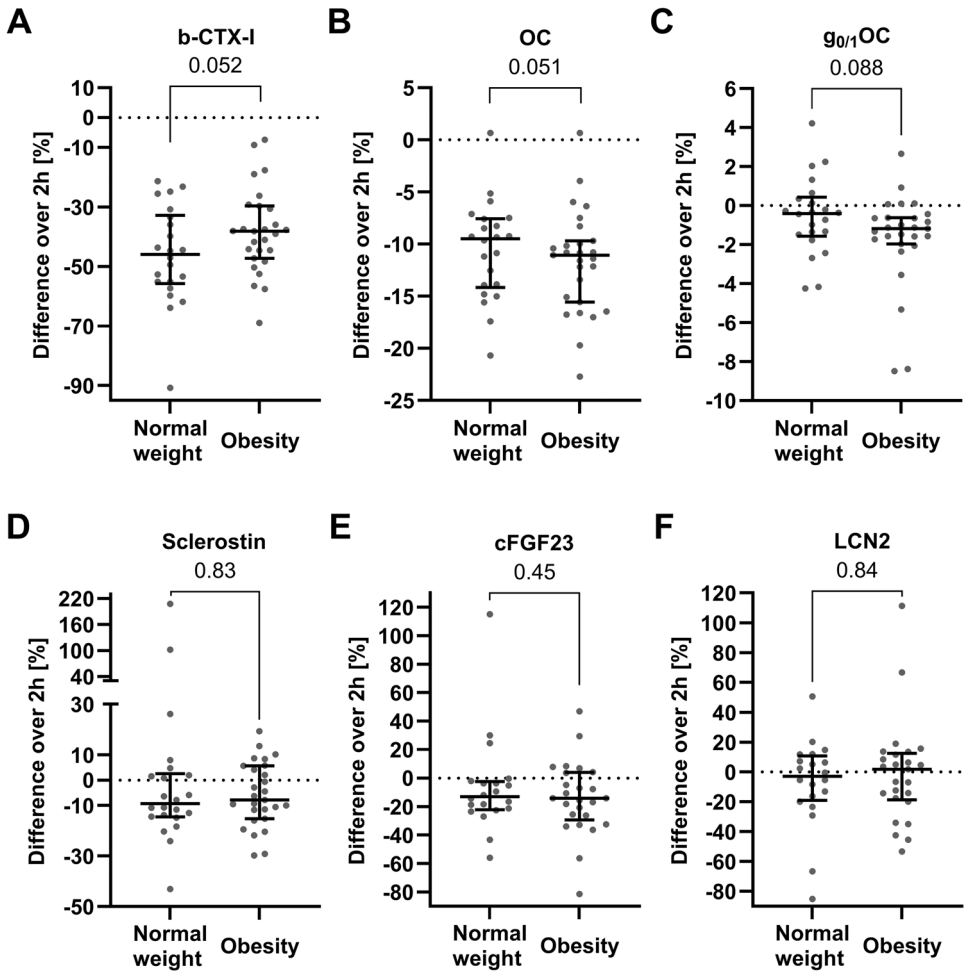

FIGURE 2 | Changes (%) in the level of bone turnover markers b-CTX-I and total OC (A, B) and osteokines $g_{0/1}$OC, Sclerostin, cFGF23, and LCN2 (C–F) over the 2 h of oral glucose tolerance test (OGTT). Data is shown as individual values with horizontal lines represent median and interquartile range. b-CTX-I, total OC, $g_{0/1}$OC, sclerostin, and cFGF23 adjusted for age, gender and group were tested with analysis of covariance. Sclerostin and cFGF23 were Ln-transformed before testing. LCN2 was tested with nonparametric Kruskal–Wallis test. Statistical significance is presented as *p*-values.

response to OGTT did not correlate with the changes in the bone resorption marker b-CTX-I ($p > 0.5$), and no correlation was found between the OGTT-induced changes in cFGF23 levels and bone turnover markers or any of the other osteokines analysed (Table 2).

4 | Discussion

In this study, we assessed the effect of a 2 h oral glucose tolerance test (OGTT) on the circulating levels of four bone-derived endocrine factors, i.e., osteokines, and evaluated whether the response to OGTT is altered in obesity. To our knowledge, this is the first comparative study on acute osteokine response to a glucose ingestion in humans with normal weight or obesity. We observed that the circulating levels of total OC, b-CTX-I, and cFGF23 were decreased in response to OGTT in the group with normal weight and with obesity, while $g_{0/1}$OC and sclerostin were significantly decreased only in the group with obesity.

Bone turnover is known to rapidly respond to glucose or nutrient intake. This is particularly prominent for bone resorption, which is significantly suppressed for a few hours after a meal [15, 23] but similar effect has also been observed after OGTT [24]. In line with the previous studies, our current data demonstrates a prominent and statistically significant decrease in the b-CTX-I levels after an OGTT. Bone formation, assessed with total OC, was also significantly decreased, even though the magnitude of the change was less pronounced.

Bone turnover is known to be suppressed in people with obesity [11, 25], and indeed, the change in bone turnover markers b-CTX-I and total OC in response to OGTT in the current study appeared to be different in the participants with obesity and in those with normal weight ($p = 0.052$ and 0.051, respectively). We have previously observed that the decrease in bone turnover marker OC during the OGTT was less pronounced in subjects with obesity compared to controls with normal body weight [11]. In contrast to the previous study, total OC decreased 16% more in the current study in the group with obesity compared to group with normal weight ($p = 0.051$). One key difference between the studies is that in the earlier study, the participants were young adults (mean age 19 yr) while in the current study OGTT was performed on skeletally mature adults (mean age 36 yr). As the skeleton matures, the rate of bone formation

TABLE 2 | Pearson's correlations for the bone turnover marker and osteokine baseline levels and for the changes in the levels over 2 h oral glucose tolerance test (OGTT).

	Correlations at baseline							
	Healthy weight				Obesity			
	b-CTX-I	Total OC	$g_{0/1}$OC	Sclerostin	b-CTX-I	Total OC	$g_{0/1}$OC	Sclerostin
Total OC	**0.61 ($p = 0.0027$)**	1			**0.80 ($p < 0.0001$)**	1		
$g_{0/1}$OC	0.33 ($p = 0.14$)	**0.76 ($p < 0.0001$)**	1		**0.61 ($p = 0.0007$)**	**0.76 ($p < 0.0001$)**	1	
Sclerostin	−0.083 ($p = 0.71$)	−0.19 ($p = 0.40$)	−0.10 ($p = 0.65$)	1	−0.14 ($p = 0.50$)	−0.072 ($p = 0.72$)	0.063 ($p = 0.75$)	1
cFGF23	0.033 ($p = 0.89$)	0.33 ($p = 0.16$)	0.40 ($p = 0.077$)	0.048 ($p = 0.84$)	0.26 ($p = 0.21$)	0.19 ($p = 0.35$)	0.20 ($p = 0.34$)	−0.18 ($p = 0.39$)
	Correlations for changes over 2 h OGTT							
	Healthy weight				Obesity			
Total OC	**0.61 ($p = 0.0025$)**	1			**0.62 ($p = 0.0006$)**	1		
$g_{0/1}$OC	0.33 ($p = 0.14$)	0.38 ($p = 0.078$)	1		−0.22 ($p = 0.27$)	−0.046 ($p = 0.82$)	1	
Sclerostin	0.13 ($p = 0.57$)	0.38 ($p = 0.083$)	**0.73 ($p = 0.0001$)**	1	0.10 ($p = 0.62$)	0.15 ($p = 0.47$)	0.25 ($p = 0.21$)	1
cFGF23	−0.089 ($p = 0.71$)	0.13 ($p = 0.59$)	−0.066 ($p = 0.78$)	−0.12 ($p = 0.62$)	0.34 ($p = 0.10$)	0.073 ($p = 0.73$)	−0.040 ($p = 0.85$)	0.17 ($p = 0.41$)

Note: Correlations are reported as r values and presented separately for the group with normal weight ($n = 22$) and for the group with obesity ($n = 27$). Statistically significant p values ($p < 0.05$) are marked in bold.

decreases more rapidly than the bone resorption [26]. It is tempting to speculate that the response to OGTT would be different in humans who have not yet reached their peak bone mass compared to those who already have surpassed it.

Furthermore, our findings demonstrate that several osteokines are also suppressed by orally administered glucose. Circulating levels of $g_{0/1}$OC, sclerostin and cFGF23 were all decreased by OGTT, while the levels of LCN2 remained unchanged, indicating that oral glucose intake can rapidly affect the release of osteokines from osteoblasts and osteocytes, which may further modulate the downstream effects of osteokines on their target organs.

While oral glucose decreased the osteokine levels, it is unclear whether the response to oral glucose is different in people with obesity than those with normal weight. One hypothesis of the current study was that people with obesity would have suppressed response in serum levels of $g_{0/1}$OC, sclerostin, and LCN2 after ingestion of glucose, since these osteokines are known to be linked to energy metabolism by regulating the pancreas and skeletal muscle, adipose tissue, and appetite, respectively [6, 7, 27, 28]. In our study, the osteokine response over the 2 h OGTT was not statistically different between the participants with normal weight and those with obesity. Osteokines thus respond to OGTT similarly regardless of body weight, which could further suggest that the endocrine functions of bone are not compromised in people with obesity.

In mice, refeeding after an overnight fast increased the serum LCN2 levels threefold [7]. In humans, LCN2 is postprandially decreased in individuals with normal weight but individuals with overweight or obesity have a suppressed response [29, 30]. Interestingly, no statistically significant changes in LCN2 levels in response to OGTT were observed in either of the study groups. In fact, we did not observe a response to oral glucose in LCN2 levels or any correlations with the levels of bone turnover markers or osteokines. Oral glucose alone may not be sufficient to affect LCN2 levels as it might require a more substantial and complex nutritional intake.

Circulating FGF23 has been shown to be decreased after OGTT in subjects with impaired glucose tolerance [31] but not in healthy subjects after ingestion partially hydrolysed starch [32]. We found that FGF23 levels decreased similarly after glucose ingestion in both groups. It is possible that the acute response of FGF23 to oral glucose is not affected by obesity as downstream effect of FGF23, is not primarily linked to energy metabolism but the homoeostasis of plasma phosphate levels.

Bone turnover markers b-CTX-I and OC correlated significantly at baseline in both study groups, indicating the coupling of bone resorption and formation at fasting condition. Additionally, baseline levels of $g_{0/1}$OC correlated with the b-CTX-I levels in the participants with obesity. Further correlations between bone turnover marker or osteokine levels at baseline were not observed. When investigating the changes in the levels of bone turnover markers and osteokines over the OGTT, a positive correlation was observed between the changes in b-CTX-I and total OC levels further highlighting the coupling of resorption and formation. Interestingly, no other correlations were

observed between the OGTT-induced changes in the group with obesity, while changes in $g_{0/1}$OC and sclerostin ($r = 0.73$, $p = 0.0001$), total OC and $g_{0/1}$OC ($r = 0.38$, $p = 0.078$), and total OC and sclerostin ($r = 0.38$, $p = 0.083$) were positively correlated in the group with normal weight. While the differences in correlations between groups were not statistically significant, it may suggest that obesity affects the ability of bone cells to respond to oral glucose. Further studies are warranted to confirm this.

The strengths of our study include clearly defined study groups with nonoverlapping BMI values, 19.3–26.2 kg/m^2 (min–max) in the group with normal weight and 28.3–51.8 kg/m^2 in the group with obesity, and inclusion of both male and female participants. However, our study also possesses certain limitations, such as the power calculations which were based on bone turnover markers due to lacking information on osteokines. In addition, the bone turnover markers and osteokines were measured only at the two timepoints (0 and 2 h) of a routine OGTT, and we were unable to study more detailed kinetics of osteokines during the OGTT. Further, our cohort consisted of relatively healthy participants with no confirmed metabolic diseases, except for obesity. In the future, it would be interesting to study osteokines in response to OGTT in individuals with type 2 diabetes or metabolic syndrome.

In summary, the current study shows that osteokines respond rapidly to oral glucose irrespective of bodyweight, indicating that osteokine signalling and endocrine functions of bone tissue are retained in obesity. Our data suggests that orally-administered glucose affects not only bone turnover but can also suppress bone endocrine functions. Furthermore, obesity may influence the effect of oral glucose on bone turnover. This emphasises the impact of nutrition on bone endocrine function and its potential role in the development of metabolic diseases.

Author Contributions

Conceptualisation: Niki Jalava, Kaisa K. Ivaska. Data curation: Niki Jalava. Formal analysis: Niki Jalava. Funding acquisition: Niki Jalava, Kirsi A. Virtanen, Kaisa K. Ivaska. Investigation: Niki Jalava, Kaisa K. Ivaska. Project administration: Pirjo Nuutila, Kirsi A. Virtanen, Kaisa K. Ivaska. Supervision: Terhi J. Heino, Kaisa K. Ivaska. Visualisation: Niki Jalava. Writing – original draft preparation: Niki Jalava, Terhi J. Heino, Kaisa K. Ivaska. Writing – review and editing: Niki Jalava, Terhi J. Heino, Pirjo Nuutila, Kirsi A. Virtanen, Kaisa K. Ivaska.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data can be shared upon request to the corresponding author.

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