Suppressed bone turnover in obesity - a link to energy metabolism? A case-control study

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Context: Observations in rodents suggest that osteocalcin (OC) participates in glucose metabolism. Based on human studies it remains unclear whether circulating OC is simply a bone turnover marker (BTM) or also a mediator in interactions between the skeleton and glucose homeostasis.

Objective: To determine the responses of BTMs, including OC, to oral glucose tolerance test (OGTT) in a case-control setting.

Design & patients: Thirty-four normoglycemic young adults (mean age 19 (SD=2.3)) with severe childhood-onset obesity and their gender- and age-matched non-obese controls underwent a standard 2-hour OGTT.

Main Outcome Measures: Glucose, insulin and six BTMs including total and carboxylated OC (cOc) were determined at baseline and at 30, 60, 90, and 120 min during OGTT.

Results: The obese and control subjects were similar in height; the mean BMIs 40.4 and 21.9 kg/m^2, respectively. HOMA index was 2.7 times greater in the obese subjects. All BTMs, except BAP, were lower in the obese subjects compared with the controls: the differences at baseline were 40%, 35%, 17%, 31% and 32% for PINP, CTX, TRACP, total OC and carboxylated OC ($p<0.05$ for all) after adjusting for whole body bone area. All BTMs decreased during OGTT. The relative values for the OGTT-responses for total, but not for cOC (measured as AUC) differed between the two groups ($p=0.029$ and $p=0.139$, respectively): the decrease in total OC during OGTT was less pronounced in the obese subjects. Responses in other BTMs were similar between the groups. No associations were observed between glucose metabolism and OCs during OGTT with linear regression.

Conclusions: Bone turnover markers were substantially lower in obese subjects compared with controls. Total OC and cOC showed less pronounced decrease during OGTT in obese subjects compared with controls, while other BTMs responded similarly in the two groups. The role of OC, if anything, in glucose homeostasis is indirect and may be mediated via other factors than glucose or insulin.

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Abbreviations:

Several lines of evidence suggest that the skeleton is integrated with energy metabolism by modulating adipocytes' insulin sensitivity and pancreatic insulin secretion (1–3). Osteocalcin (OC), a bone-specific protein, is shown to promote insulin secretion and positively regulate energy metabolism in mice (4). Several earlier studies have used serum OC as a biomarker of bone turnover and evaluated OC as a surrogate marker for fracture risk and osteoporosis (5, 6). OC undergoes vitamin-K-dependent carboxylation that confers greater affinity to bone matrix. A portion of OC, however, remains uncarboxylated and this uncarboxylated form of OC (ucOC) is proposed to be a mediator in the endocrine loop (7, 8). Further, administration of ucOC has been shown to decrease the severity of obesity and type 2 Diabetes (T2DM) in mice fed with high-fat diet (9). Release of ucOC may be regulated by insulin signaling directly in the osteoblasts (10) or indirectly via osteoclastic bone resorption and release of ucOC from bone matrix (11), at least in mice.

It is unclear if a similar regulatory system is present in humans (12, 13). In cross-sectional studies in the elderly (14, 15), young adults (16) and obese subjects (17) fasting plasma glucose, insulin and insulin resistance were inversely related to OC. Higher serum OC levels were measured in normoglycemic subjects than in subjects with insulin resistance (14, 18), but the association has not been observed in all studies (19, 20). Further, suppression of bone turnover does not appear to associate with glucose metabolism in humans (21). Even if some associations have been observed between OC and energy metabolism in humans, cross-sectional studies do not prove causality and therefore the role of OC in glucose metabolism in humans remains unestablished (12).

Obesity may have detrimental effects on bone metabolism through inflammatory cytokines, adipokines, and free fatty acids (22). Even the role of leptin in bone metabolism seems conflicting: a direct stimulatory effect on bone and an indirect inhibitory effect via the central nervous system (CNS) have been described (23). Patients with T2DM have increased fracture risk (24); animal and cell culture studies have shown that abnormal or disturbed glucose metabolism also impairs bone formation (25). Dimitri et al (2012) concluded that beneficial and unfavorable effects of obesity may vary with age (26). They also emphasized the need for other than densitometric data to fill in the gaps in our understanding on obesity and bone.

Early-onset obesity is characterized by severe weight gain already before puberty and associates with several metabolic disturbances including impaired glucose tolerance (27). Prepuberty and puberty are critical periods for bone development (28). We hypothesized that severe obesity during childhood has long-term implications for bone health. The objectives of the present study were firstly, to compare multiple bone turnover markers between obese subjects with childhood-onset obesity and age- and sex-matched normal-weight controls. Secondly, we evaluate the response of bone turnover markers (BTMs), including OC, to a rapid increase in circulating glucose levels, and the interplay between glucose, insulin and BTMs, during an oral glucose tolerance test (OGTT).

Materials and Methods

Subjects

This study was designed to assess the skeletal and metabolic characteristics of severe childhood-onset obesity and was carried out at Children's Hospital, Helsinki University Central Hospital, Finland. An ethical approval was obtained from the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa. Informed written consent was obtained from all study participants. Inclusion criteria for the obese subjects were: i) weight-for-height ratio exceeding 60% before age 7 years, according to Finnish growth standards, ii) referral because of severe obesity to Children's Hospital, Helsinki University Central Hospital, during childhood, iii) at the age of 7 years lived in the capital region of Helsinki, and iv) aged between 15 and 25 years at the time of the study. Altogether 230 patients fulfilling these inclusion criteria were identified in the Children's Hospital patient register. All were invited to participate in the study and 42 (18%) eligible subjects consented. All participating patients had been followed by a pediatrician at Children's Hospital and common endocrine and genetic causes of obesity had been excluded (eg, Prader Willi syndrome, pseudohypoparathryoidism, hypercortisolism, hypothyroidism). They are referred to as 'obese subjects' in the text. Before study visit two obese subjects withdrew their consent due lack of time, one due to pregnancy, and one due to diagnosis of T2DM. In addition, OGTT was incomplete in three obese subjects and these data were not included in the analysis. For each patient an age- and sex matched control was selected from the civil register. Sampling of controls was limited to the capital region of Helsinki. Exclusion criteria for the controls were obesity (weight-for-height ratio above 40%) before age 10 years. Altogether 35 controls consented our study during 2011, but in one subject OGTT was incomplete. Altogether 34 age- and sex matched patient-control pairs, who underwent OGTT and had complete data, were included in the present study.

Anthropometry including height, weight, waist and hip circumferences was collected during the study visit. Weight was measured in light clothing with Seca digital scale (www.seca.com) to the nearest 0.1 kg. Height was measured with a fast stadiometer connected to the scale to the nearest 0.1 cm. Waist circumference was measured to the nearest 0.1 cm using a non-stretch narrow tape at the middle of the abdomen between the ribs and the iliac crest at the end of expiration. Hip circumference was measured at the widest part of the buttock, at the great trochanters. Body mass index, BMI was calculated as weight divided with square height, in kg/m^2.

Background data on smoking, fractures and physical activity was collected with a questionnaire. Recent history of physical

activity (12 months retrospective) consisted of school/work trips, guided activity in free time, and free time activity on their own and was expressed as min/d.

Oral glucose tolerance test, OGTT

After an overnight fast of at least 10 hours all subjects started a standard 2-hour OGTT between 8.30 and 9.30 am. A cannula was inserted in an antecubital vein for blood sampling and baseline (0 minutes) blood samples were drawn. For OGTT, the study subjects ingested 75 g of glucose (Diasol®, dissolved in 400 ml of water) within 5 minutes. Blood samples were drawn at 30, 60, 90, and 120 minutes after glucose load.

Laboratory measurements

Blood samples were obtained for glucose, insulin and BTMs (detailed below) at all time-points. Glucose was analyzed by spectrophotometric hexokinase and glucose-6-phosphate dehydrogenase assay (Gluko-quant glucose/hexokinase, Roche Diagnostics) with a Hitachi Modular automatic. Impaired fasting glucose was defined according to WHO as plasma glucose concentration > 6.1 but ≤ 7.0 mmol/l at 0 minutes and as impaired glucose tolerance ≥ 7.8 mmol/l but < 11.1 mmol/l at 120 minutes. Diabetes was defined as plasma glucose concentration ≥ 7.0 mmol/l at 0 minutes and/or ≥ 11.1 mmol/l at 120 minutes (29). Based on OGTT findings, all subjects were considered normoglycemic. After 30 minutes serum samples were centrifuged and divided into aliquots and stored at – 80°C for further analyses.

Serum insulin was measured with time-resolved immunofluorometric assay (Perkin Elmer Life Sciences, Finland) with a detection limit of 0.5 mU/l and an interassay CV less than 4%. The insulin-resistance index determined by homeostasis model assessment (HOMA-IR) was calculated as the product of the fasting serum insulin concentration (in mU/l) and fasting plasma glucose concentration (in mmol/l) divided by 22.5 (30). The glycosylated hemoglobin (HbA1c) was measured by photometric immunoassay.

Bone-specific alkaline phophatase (BAP), intact N-terminal propeptides of type I collagen (PINP) and C-terminal cross-linked telopeptides of type I collagen (CTX-I) were measured with automated methods using the IDS-iSYS automated analyzer (IDS Ltd, Boldon, UK), and tartrate-resistant acid phosphatase isoform 5b (TRACP 5b) using a manual assay (BoneTRAP(R), IDS Ltd). All BTM measurements were performed by ValiRx Finland Ltd (Oulu, Finland). Serum total OC and carboxylated OC (cOC) were determined by two-site immunoassay protocols (31), as described in detail previously (32). All samples were measured simultaneously at the end of the study in doublets. To illustrate the differences in bone metabolism between the groups bone formation (PINP/BAP) and resorption (CTX/TRACP5b) indexes were calculated at baseline (33, 34) (Table 2). Additionally, a turnover index (PINP/CTX) was calculated to reflect coupling of bone turnover (35).

Serum-25-OH Vitamin D (S-25-OHD) concentration was analyzed by liquid chomatography in tandem with mass spectrometry (LC-MS) and intact parathyroid hormone (iPTH) with an immune chemiluminometric assay. Serum adiponectin was determined with Human Total Adiponectin/Acrp30 Quantikine ELISA Kit and serum leptin with Human Leptin R Quantikine ELISA Kit (R&D Systems, Minneapolis, USA) with intra- and interassay CV of $< 12\%$.

Other measurements

Whole body bone area (WB BA) was measured with Lunar Prodigy Advance DXA. Calibration of the measurement was performed with a spine phantom; inter-CV% for BA was 0.38%. Reducibility of DXA measurement for total body is: BMD = 0.63%, BMC = 0.45% and BA = 0.78% (36). Four obese subjects exceeded in weight 160 kg which is the maximum weight for the DXA device and the mean WB BA value for all obese subjects (2570 cm^2) was used for them in multivariate data analyses.

Statistics

Differences in baseline characteristics between groups were tested with Independent Samples *t* test. If variables were not normally distributed logarithmic transformations were performed. Differences in BTMs between the groups were tested with MANCOVA when adjusting for WB BA.

Response to OGTT was tested with repeated measures ANOVA and comparison of effect between groups was performed with contrasts. For each variable mean value (SD) is given or in case of adjustments, mean (SEM). Relative values were calculated by dividing absolute values with the baseline value. These are presented only for total OC and cOC to elucidate the differences in response to OGTT between groups.

At baseline determinants for BTMs were tested with linear regression forward method. Potential predictors included in the model were patient status, gender, age, glucose, insulin, iPTH,

Table 2. Spearman correlation between leptin/adiponectin and bone turnover markers/hormones at baseline

GROUPS COMBINED		BAP, μg/liter	PINP, ng/ml	CTX, ng/ml	TRACP5b, U/liter	TotOC, ng/ml	cOC, ng/ml	Insulin, mU/liter	Glucose, mmol/liter	PTH, ng/liter	25-OHD, nmol/liter	Adiponectin, ng/ml	Leptin, pg/ml
Leptin, pg/ml		−0.184	−0.465	−0.587	−0.434	−0.505	−0.502	0.635	0.175	0.388	−0.305	−0.304	1.000
	P-value	0.153	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	0.156	0.001	0.011	0.012	
Adiponectin, ng/ml		−0.399	−0.249	−0.066	−0.063	−0.119	−0.136	−0.568	−0.373	−0.155	0.216	1.000	−0.305
	P-value	0.001	0.051	0.608	0.626	0.356	0.295	<0.001	0.002	0.211	0.077		0.011
OBESE SUBJECTS													
Leptin, pg/ml		−0.453	−0.605	−0.684	−0.602	−0.596	−0.560	0.320	0.056	0.457	−0.399	−0.190	1.000
	P-value	0.012	<0.001	<0.001	<0.001	<0.001	0.001	0.061	0.748	0.029	0.040	0.274	
Adiponectin, ng/ml		−0.235	−0.108	0.035	0.114	0.077	−0.020	−0.546	−0.161	−0.012	−0.043	1.000	−0.190
	P-value	0.212	0.570	0.853	0.548	0.685	0.917	0.001	0.356	1.049	1.182		0.274
CONTROLS													
Leptin, pg/ml		−0.440	−0.404	−0.523	−0.081	−0.422	−0.402	0.046	−0.231	0.011	0.194	0.259	1.000
	P-value	0.012	0.022	0.002	0.659	0.016	0.025	0.802	0.203	0.952	0.278	0.145	
Adiponectin, ng/ml		−0.387	−0.490	−0.323	−0.400	−0.418	−0.452	−0.437	−0.415	−0.014	0.173	1.000	0.259
	P-value	0.028	0.004	0.071	0.023	0.017	0.011	0.012	0.018	0.940	0.336		0.145

S-25-OHD, leptin and adiponectin. Spearman's correlation was used to test association between leptin and BTM/endocrine factors. Analyses were performed in groups combined and repeated after stratification into patient status.

Association of glucose and insulin with BTMs was tested with linear regression. Outcomes were changes in BTMs from baseline to 30 minutes ($\Delta = BTM_{30\ minutes} - BTM_{0\ minutes}$; reflecting an acute response), to 60 minutes (steady state) and area under the curve (AUC) for overall response, while the exposures were corresponding changes in glucose, insulin, glucose adjusted for insulin and insulin adjusted for glucose. Models included additional adjustments for gender and patient status as all BTMs were significantly lower in females and obese subjects than in males and controls, respectively. For each association the standardized beta, p-value and adjusted R^2 values are presented. AUCs were calculated with GraphPad Prism version 5.0.

All analyses were carried out using version 19.0 of the IBM SPSS Statistics.

Results

Baseline characteristics

Baseline characteristics for the groups are presented in Table 1. The mean age of the participants was 19.4 years and 59% of all subjects were females (Table 1). Heights were comparable but other anthropometric variables were significantly greater in obese subjects, mean BMIs being 40.4 kg/m^2 for the obese subjects and 21.9 kg/m^2 for the controls. Altogether 55% of the obese subjects and 3% of the controls had abnormally high fasting insulin concentration for this population (>12 mU/l) (37) indicating increased insulin resistance in obese subjects, but none were diagnosed with T2DM. As expected the obese subjects had lower adiponectin, but higher leptin concentrations. Serum 25-OHD concentrations did not differ between the groups, but the obese subjects had significantly higher iPTH (57.6 pg/ml vs 39.1 pg/ml, $P = .003$) (Table 1). Smoking was more frequent in obese subjects than in controls ($P = .022$).

Bone turnover markers

At baseline the determinants for BTMs, were tested with linear regression in the entire cohort and after stratification for patient status. In the entire cohort, the gender, leptin and adiponectin were the significant predictors of BTM explaining from 22 to 32% of the variation (Supplementary Table 1). In general, concentrations of all BTMs were higher in men than in women. In the normal-weight controls gender explained most of the variation, whereas leptin was the main determinant in the obese subjects. Associations of adipokines with BTMs and hormones were tested with Spearman correlation (Table 2)

Table 1. Characteristics of age- and sex-matched subjects with mean (SD)

	OBESE SUBJECTS	CONTROLS	P-value	Adjusted for WB BA P-value
N	34	34		
Male, %	41	41		
Age, y	19.3 (2.3)	19.4 (2.3)	0.860	
Height, cm	173.3 (8.6)	171.7 (11.5)	0.525	
Weight, kg	120.9 (27.7)	64.8 (12.1)	<0.001	
BMI, kg/m^2	40.4 (9.4)	21.9 (3.1)	<0.001	
Waist circumference, cm	114.3 (15.9)	72.7 (8.1)	<0.001	
Hip circumference, cm	125.8 (15.3)	97.3 (6.5)	<0.001	
Waist-to-hip ratio	0.91 (0.09)	0.75 (0.06)	<0.001	
Smokers, %	36.7	12.2	0.022	
Fractures, %	39.4	47.1	0.527	
Physical Activity, min/d	42 (59)	45 (30)	0.592	
HbA_{1C}, %	5.30 (0.31)	5.17 (0.23)	0.047	
Insulin, mU/liter	15.69 (9.43)	6.31 (3.05)	<0.001	
HOMA	3.89 (2.36)	1.46 (0.77)	<0.001	
iPTH, ng/liter	57.6 (28.1)	39.2 (19.1)	0.003	
25-OHD, nmol/liter	62.8 (30.3)	71.5 (24.1)	0.196	
Adiponectin, ng/ml	7734 (3115)	11625 (6499)	0.004	
Leptin, pg/ml	51887 (28990)	8875 (6995)	<0.001	
WB bone area, cm^2	2573 (318)	2303 (287)	0.001	
PINP, ng/ml	82.0 (43.1)	121.4 (110.8)	0.036	0.05
CTX, ng/ml	0.73 (0.36)	1.02 (0.64)	0.042	0.012
BAP, μg/liter	20.4 (7.3)	22.4 (16.2)	0.542	0.281
TRACP5b, U/liter	3.41 (0.80)	4.16 (1.19)	0.005	0.02
Total OC, ng/ml	13.61 (5.21)	17.85 (9.62)	0.048	0.022
cOC, ng/ml	13.14 (5.07)	17.49 (9.36)	0.039	0.023
Ratio of PINP to CTX	116.20 (28.50)	111.80 (34.70)	0.584	0.419
Formation index[1]	4.14 (1.67)	5.32 (1.68)	0.01	0.021
Resorption index[2]	0.21 (0.07)	0.25 (0.12)	0.186	0.031

HOMA, homeostatic model assessment; WB, whole body; PINP, procollagen type 1 amino- terminal propeptide; CTX, collagen type 1 cross-linked C-telopeptide; OC, osteocalcin; cOC, carboxylated osteocalcin

accordingly: leptin was inversely related to all BTMs in the obese subjects, to all except for TRACP5b in the control subjects, and in the combined cohort, to all BTMs except for BAP. Adiponectin was not related to any BTMs in the obese subjects but inversely related to all BTMs except for CTX in the control group. In combined cohort adiponectin was only related to BAP and tended to relate with PINP. Smoking and physical activity were not confounders for BTMs (data not shown).

Obese subjects had on average an 11.7% greater WB BA compared with normal-weight controls (Table 1) ($P = .001$). Despite this, BTMs (except for BAP) were significantly lower in obese subjects compared with controls at baseline (Table 1) and this was confirmed after adjusting for WB BA in MANCOVA: obese subjects had 40%, 35%, 17%, 31% and 32% lower concentration of PINP, CTX, TRACP5b, Total OC and cOC, respectively ($P < .05$ for all) (Figure 1).

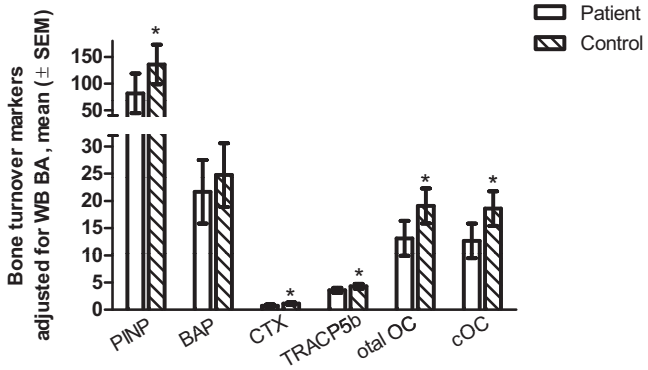

Figure 1. Bone turnover markers in two groups at baseline after adjusting for whole body bone area. Values are given as mean (SEM). Unit for [1]PINP,CTX, Total OC and cOC is ng/ml, for [2]BAP μg/l and for [3]TRACP5b U/l.

The bone formation index was higher in controls than obese subjects while no difference in other indexes were observed between the groups (Table 1). Taking into account WB BA in MANCOVA confirmed findings on low formation and resorption indexes in obese subjects.

Responses during OGTT

As expected, both plasma glucose and serum insulin concentrations peaked at 30 minutes after ingestion of the 75-g glucose load in both groups (Figure 2). Mean plasma glucose concentrations were similar in the groups at any time point (AUC in repeated measures ANOVA; $P = .066$), whereas insulin response to OGTT differed (AUC in repeated measures ANOVA; $P = .003$); 3-fold higher serum insulin concentrations were observed in obese subjects compared with controls.

An interaction with time was observed in both insulin and glucose (both $P < .001$): after 30 minutes both values gradually decreased towards the baseline values. At the 2-hour time point glucose values were lower than 7.8 mmol/l in all subjects. There was a significant change in all BTMs during OGTT. The decrease was most consistent for CTX and OC (Figure 2 and 3). An acute response in all BTMs was noted already at 30 minutes and the maximal suppression was observed at 60 to 90 minutes. The max-

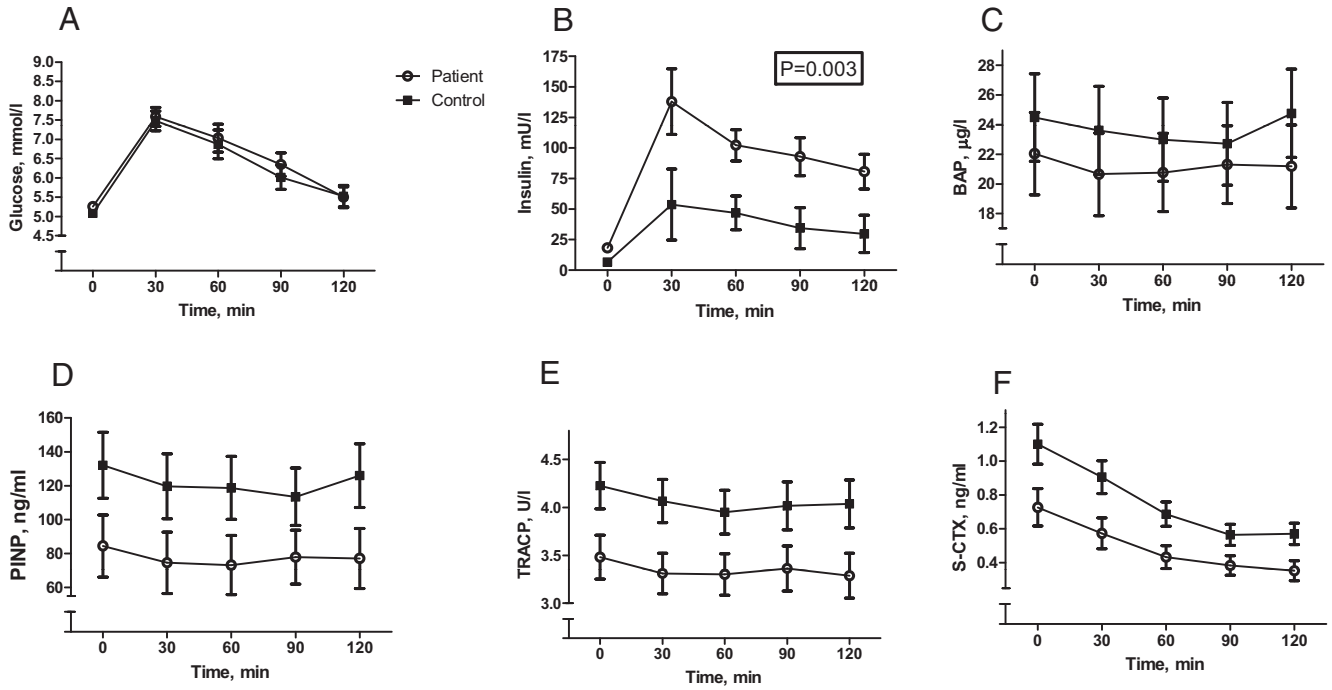

Figure 2. Responses of A) glucose, B) insulin, C) BAP, D) PINP, E) TRACP 5b and F) CTX to OGTTt in obese (open circles) and in control subjects (black squares). Values are given as mean (SEM). The response in insulin differed between the groups ($P = .003$). BTMs were significantly lower at baseline in obese subjects compared with controls, but they responded similarly during OGTT.

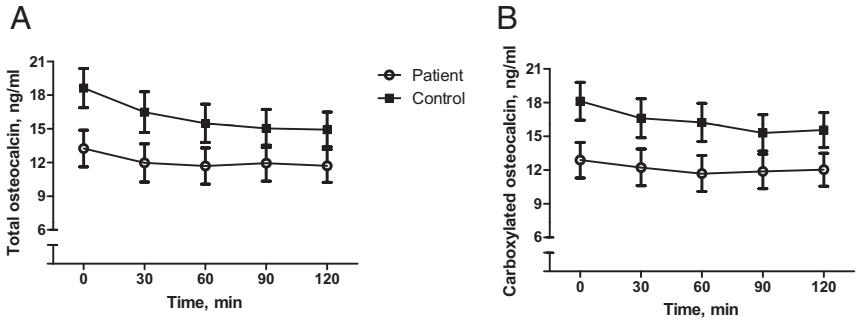

Figure 3. Response of A) total and B) carboxylated osteocalcin during OGTT in obese (open circles) and in control subjects (black squares). Values are given as mean (SEM). Multivariate analysis revealed an interaction with time and group for total OC ($P = .029$) and cOC ($P = .046$).

imum decreases from the baseline during OGTT for CTX, PINP, TRACP5b and BAP were 52%, 11%, 5% and 6%, respectively. These decreases were all statistically significant ($P < .02$) and similar in the groups (p-values varied between 0.5 and 0.7 for the comparison of groups).

During OGTT total OC and cOC acted somewhat differently in groups (Figure 3): the maximum decreases in obese subjects were 13.1% and 10.9% for total OC and cOC, respectively while the corresponding reductions in the control group were significantly higher 21.1% ($P = .022$) and 16.5% ($P = .055$), respectively. However, the relative decrease in total OC during OGTT was less pronounced in obese subjects than in controls ($P = .029$), while there was no difference between groups in cOC ($P = .139$) (Figure 4).

Linear regressions

Associations of glucose and insulin concentrations with BTMs at different time points are presented in (Supplemental Table 2). Acute and steady-state responses of glucose and BAP were inversely associated; the association remained significant also after adjusting for insulin. Similarly acute change in glucose was inversely related to overall response (AUC) in CTX, however, the significance for this association was at borderline ($P = .059$). Acute, steady state or overall responses of glucose or insulin alone or together with glucose did not associate with other BTMs at any time point.

Discussion

Present study shows that obese subjects with early-onset severe obesity had a substantially lower bone turnover markers (except for BAP) compared with sex- and age-matched controls. Several other reports support our findings (38, 39), but also conflicting results, though based only on few BTMs, have been presented (40–42). In general, obesity is associated with greater WB BA, which in turn can be expected to associate with higher concentration of BTMs: for example male subjects typically have higher concentrations of BTMs than females (43). However, this projection does not directly apply to the obese subjects because adjustment for skeletal size increased the differences and confirmed supressed bone turnover rate, assessed by serum markers, in the obese subjects vs controls.

The response of multiple BTMs ie, BAP, PINP, CTX and TRACP5b, to OGTT were comparable between the obese subjects and controls: bone turnover was markedly suppressed during a standard 2-hour OGTT. The most pronounced decrease, – 52% from baseline, was noted for CTX. Between the groups, the total OC, but not cOC, responded differently than the other BTMs during OGTT: the decrease was less pronounced in obese subjects than in controls. This implies that the response of OC differs from other BTMs. It also confirms the previous findings indicating that OGTT-induced changes in BTMs may be potentially mediated by different, nonskeletal mechanisms (32).

A decrease in BTMs occurred already at 30 minutes time-point while the maximum suppression was observed at 60 or 90 minutes, depending on the marker. Only limited number of studies has utilized the same study design in this respect and their results are in accordance with ours (32, 44). Although response of total OC during OGTT differed between the groups, no associations were observed between glucose and/or insulin and OC in a regression analysis. The observed inverse associations between the glucose levels and BAP or the glucose and CTX imply that postprandial or OGTT-induced hyperglycemia in general suppresses bone turnover. In accordance, earlier studies have suggested hyperglycemia to contribute to lower bone turnover (25) and to favor differentiation of mesenchymal stem cells into adipocyte lineage (45). The unique response of OC to OGTT could have a logical explanation as it is considered a secondary marker of late stages of bone turnover: OC is produced by mature osteoblasts during mineralization process to stabilize the structure of bone mineral, the hydroxyapatite (46). In mice, deletion of insulin receptor specifically in osteoblasts has been demonstrated to result in decreased secretion of OC (47). Thus, delayed decline in OC noted in obese subjects could result from suppressed bone turnover at baseline, which in turn is further suppressed during OGTT.

Although none of our subjects were considered diabetic, over half of the obese subjects presented with increased insulin resistance as indicated by abnormally high

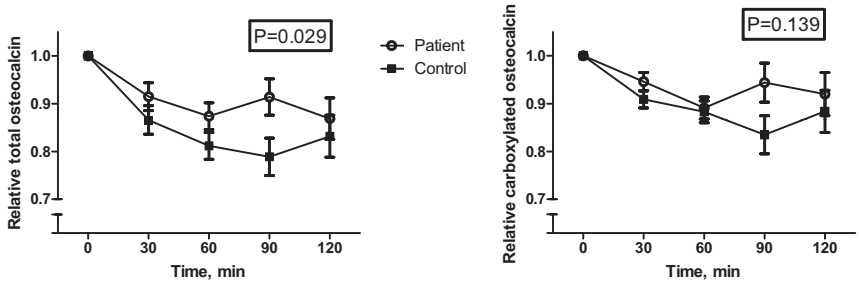

Figure 4. Relative changes in A) total and B) carboxylated osteocalcin during OGTT in obese (open circles) and in control subjects (black squares). Values are given as mean (SEM). AUCs differed between the groups in total ($P = .029$), but not in cOC ($P = .139$).

fasting insulin levels and a higher HOMA-IR index. During OGTT, insulin concentrations were on average three fold higher in obese subjects compared with those in controls whereas the fasting glucose, glucose during OGTT and post-OGTT glucose did not differ between the groups. All subjects were thus considered normoglycemic with no OGTT-defined IGT despite indicators of increased insulin resistance and general obesity known to be a major risk factor to IGT (27). We observed significantly lower total OC and cOC concentrations in obese subjects compared with normal weight controls at baseline in line with earlier observations (14, 15, 17, 18).

Bone formation index (PINP/BAP) was lower in the obese while no difference in other calculated indexes were seen between the groups. These findings suggest that osteoblasts are less active per se and produce less collagen, but osteoclasts are as functional in obese subjects as in controls. In line with our findings, Sinha et al (2011) reported that bone remodelling was markedly stimulated after a bariatric bypass operation (in a follow up study) despite aggressive postoperative supplementation with calcium and vitamin D (48). Correspondingly, premenopausal women with high trunk fat mass had decreased bone formation together with inferior bone quality at tissue level (39) in a study in which a reduction in serum resorption markers was noted; some other studies do not support this hypothesis (41, 42).

The overall bone remodelling is regulated by cytokines and hormone interactions. We observed higher iPTH levels in obese similar to Grethen et al (42) although we found no association between iPTH and BTMs (data not shown). Both groups had, taking into account the local reference range, on average sufficient vitamin D status (>50 nmol). Vitamin D deficiency could contribute to elevated iPTH and associate with enhanced bone turnover, opposite to our findings. We speculate that the underlying mechanism for lower bone turnover rate relates to leptin. Some earlier studies have shown similar findings (19, 41, 42, 49). In our study leptin concentration was a significant determinant for baseline BTMs. Leptin is secreted by adipocytes and our obese subjects had obviously greater whole body fat mass than the normal-weighted controls. Leptin correlated inversely with all BTMs in the whole cohort but the correlations were even more pronounced in obese subjects who had on average 5.8 fold higher leptin concentration than the controls.

Remodelling of bone is a continuous process that maintains the integrity of the skeleton while it adapts the skeleton for loading and releases minerals, and cytokines/hormones for nonskeletal purposes (1). Low bone turnover in relation to higher skeletal area in young subjects is an intriguing finding which may have multiple consequences. The greatest concern is the indicated fracture risk: several studies have shown that overweight or obese children are overrepresented in pediatric fracture patients (50–52). A low bone formation rate in premenopausal women with idiopathic osteoporosis was marked with deteriorated microarchitecture and lowered stiffness evaluated from bone biopsies with microCT (39).

The strengths of our study include a comprehensive analysis of bone metabolism by measuring multiple BTMs and different forms of OC at various time-points during OGTT in obese young subjects vs controls, which to our knowledge is a novel study design to explore the causality between bone turnover and energy metabolism in a population without significant comorbidities. Our relatively small study has several limitations. A standardized OGTT does not allow us to study the continuous direct effects of glucose or insulin on bone metabolism. Feeding with an acute glucose dose gives rise to various carbohydrate-induced incretin hormones such as GIP and GLP-2 which might confound our findings. GLP-2 has been identified to directly inhibit bone resorption (53–55) and our results agree with these findings as the CTX values were markedly suppressed during OGTT. A further limitation is that we measured only total adiponectin and not the isoforms which associate more strongly with insulin resistance and metabolic syndrome. On the other hand, OGTT differs from a more physiological intake of nutrients, but otherwise corresponds to real life situation only with few confounding factors. Further, we did not have data on dietary intake of vitamin K in our population. Even if carboxylation of glutamic acid residuals in osteocalcin is vitamin K dependent (12), it is unlikely that any of our subjects had severe vitamin K deficiency since they were not on parenteral feeding or had any other dietary restrictions. We did not directly measure ucOC but assessed total OC and cOC

and estimated the biological activity of OC in the study cohort.

We conclude that young obese subjects with signs of increased insulin resistance have lower concentrations of BTMs other than BAP compared with normal weight subjects at baseline. Glucose intake suppressed bone turnover during OGTT and various BTMs, except total OC, responded similarly in the groups. The postprandial total OC was suppressed but the OGTT-induced effect appeared later in obese subjects than in controls. Therefore our findings do not directly support the previously hypothesized presence of a short-term feedback loop between OC and energy metabolism in humans even if the role of OC in long-term regulation of energy metabolism cannot be excluded. The potential clinical relevance of these findings remains to be evaluated in future studies.

Acknowledgments

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