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Weight loss-induced increases in osteocalcin are associated with improvements in glucose homeostasis

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Abstract

Background: Recent data suggest that the weight loss-associated increase in the osteoblast-specific peptide osteocalcin (OC) might be related, not to increased bone formation, but to the endocrine action of OC enhance to both pancreatic insulin secretion and insulin sensitivity of target tissues. Thus, the purpose of the present study was to examine the possible role of serum OC as a regulator of glucose homeostasis following weight loss in sedentary overweight or obese women.

Methods: This study was a post hoc analysis of three independent weight-loss intervention studies, which varied in weight-loss magnitude and duration. Serum glucose, insulin, bone formation (OC and bone-specific alkaline phosphatase, BAP) and resorption (C-terminal peptide of type I collagen, CTX) markers were measured before and after weight loss in sedentary overweight or obese women (n=77) and were compared with data from active, lean controls (n=46).

Results: Fasting insulin, glucose and HOMA-IR significantly improved with weight loss. OC and CTX increased significantly following weight reduction, while BAP remained unchanged. The percent increase in OC was positively associated with the magnitude of the weight loss (r=0.25, p=0.02), while the increase in CTX was not (r=0.10, p=0.26). Following weight loss, serum OC was negatively associated with fasting glucose (r= -0.232, p=0.02), and weight-loss-associated changes in serum OC were positively correlated with changes in HOMA2-%B (r=0.33, p=0.04).

Conclusions: These preliminary results suggest that OC might play a role in the improvements in glucoregulation observed following weight reduction in overweight, sedentary women.

Keywords: Osteocalcin; Bone-specific alkaline phosphatase; C-terminal peptide of type I collagen; Weight loss; Glucose homeostasis; Insulin resistance

Introduction

Insulin resistance, an impaired response of target tissues to circulating insulin, is a key component of the metabolic syndrome [1]. Obesity, in particular an increase in visceral adipose tissue mass, exacerbates insulin resistance through a variety of mechanisms, including secretion of adipokines [2] that impair insulin sensitivity in other target tissues, such as skeletal muscle, liver, and pancreas [3]. Very recent evidence suggests that bone, like adipose tissue, is an endocrine organ that participates in the regulation of glucose homeostasis [4]. The osteoblast-specific peptide osteocalcin (OC) increases insulin secretion, enhances insulin sensitivity in the liver, adipose tissue and skeletal muscle, and reduces accumulation of adipose tissue in genetically modified animals [4,5].

In our previous studies of the effects of weight reduction on bone mass and turnover in overweight adults, we have consistently observed that weight reduction increases serum osteocalcin (OC), which is widely used as a serum marker of bone formation [6]. In independent weight-loss intervention studies that varied in both duration and magnitude of weight reduction, we observed increases in OC ranging from ~15 to 40% [7-9]. However, explaining these observations has been difficult for two reasons: bone formation, as measured by serum markers, is decreased by both acute and chronic negative energy balance [10,11]; and, bone mass is reduced with weight loss [12]. Recent data suggest that the increases in OC observed with weight loss might be related, not to increased bone formation, but to the endocrine function of OC as a hormone that increases pancreatic insulin secretion and insulin sensitivity of target tissues [4,13]. Thus, the purpose of the present study was to investigate whether the increase in OC observed following weight loss in overweight women might be related to its function as a regulator of glucose homeostasis.

Materials and Methods

Study design and study participants

The present study is a post hoc analysis of data collected during three previously published weight-loss intervention studies [8,15]; only the women (n=77) who participated in the earlier studies were included in this secondary data analysis to avoid confounding by potential sex differences. In addition, data from active, lean women (n=46) were included as a normal reference ([14]; and our unpublished data). Thus, the total sample (n=123) included both sedentary overweight or obese subjects (n=77) and active, lean controls (n=46); the characteristics of the study participants are shown in (Table 1). All procedures involving human subjects were in accordance with the ethical standards of the University of Missouri Institutional Review Board, and with the Helsinki Declaration of 1975 as revised in 1983. Informed written consent was obtained from each subject prior to participation.

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The duration and magnitude of the weight-loss differed among studies, as did participant body mass index (BMI) prior to weight reduction, as shown in (Table 1). In each study, weight loss was achieved via a combination of energy restriction and increased exercise-related energy expenditure (e.g., brisk walking), as previously described [8,9,15].

Outcome measures

Body weight, measured to the nearest 0.05 kg, and height to the nearest 0.5 cm, were used to calculate body mass index (BMI, kg/m^2). Body composition, i.e., percent body fat, was measured via whole body DXA scans, as previously described [8,9,15]. Blood was collected in the early morning after both an overnight fast and at least a 24-h abstention from exercise using a butterfly needle inserted into the antecubital vein with the subjects in the seated position. Serum and plasma were separated by centrifugation at 4°C for 15 minutes at 2000g in a Marathon 2100R centrifuge (Fisher Scientific, Pittsburgh, PA, USA) and stored in cryogenic vials at -80°C. The concentrations of OC and bone-specific alkaline phosphatase (BAP) in serum were measured using commercially available ELISA kits (Quidel, San Diego CA, USA). BAP served as a negative control, as, like OC it is a serum marker of bone formation [6], but it is not known to regulate insulin sensitivity. The anti-OC antibody used in the ELISA recognizes only intact OC; therefore, because the antibody does not bind OC fragments, which are released during bone resorption, OC measured using this ELISA results only from de novo synthesis. Cross reactivity of the anti-human bone-AP antibody with liver AP is 3-8% and with intestinal bone-AP is 0.4%. We also measured serum C-terminal peptide of type I collagen (CTX) as a marker of bone resorption using commercial ELISA (Immunodiagnostic Systems, Inc., Scottsdale, AZ, USA). All OC, BAP, and CTX assays were performed in the same laboratory in serum samples that had not been previously thawed with intra-assay coefficients of variation (CV) of 3.0%, 4.6%, and 6.4%, respectively. The concentrations of glucose and insulin in plasma were measured using a commercially available colorimetric assay (Thermo, Arlington, TX, USA) and a chemiluminescent immunoassay (Immulite 1000, Siemens, New York, NY, USA), respectively. The homeostasis model assessment of insulin resistance (HOMA2-IR), β-cell function, and insulin sensitivity were calculated from fasting glucose and insulin concentrations, as previously described [16,17]. Both β-cell function (HOMA2-%B) and insulin sensitivity (HOMA2-%S) are expressed as percentages relative to a normal, reference population (100%).

Statistics

Data were analyzed using SPSS statistical software (SPSS/11.0, SPSS, Chicago, IL, USA) and statistical significance was set at $p \leq 0.05$ for all tests. Because the weight-loss interventions differed in magnitude and duration and in participants' baseline BMI, the effect of weight loss on outcome measures was independently evaluated for each study using a one-factor (time, i.e., weight loss) repeated measures ANOVA. Comparisons among weight-loss groups and lean controls were performed using a one-way ANOVA with post hoc least-significant difference (LSD) pair-wise comparisons. Pearson's correlation was used to examine relationships between absolute concentrations and weight-loss-associated changes (%) in body weight, OC, BAP, CTX, glucose, insulin, HOMA2-IR, HOMA2-%B, and HOMA2-%S (n=77). Data are means ± SEM.

Results

Anthropometric characteristics of the research participants are shown in (Table 1). Participants were overweight or obese prior to weight loss, which ranged in magnitude from 5 to 19% of initial body weight. Fasting glucose, insulin, and HOMA2-IR were significantly greater in overweight or obese subjects compared with lean, physically active controls, while the lean participants had higher HOMA-S% (Table 2). Following weight reduction, fasting glucose and insulin concentrations decreased significantly (Table 2).

Pre-weight-loss serum concentrations of OC and BAP in overweight or obese participants were less than lean controls (Table 3). Following weight reduction, OC increased significantly, but remained lower than OC in lean controls (Table 3); CTX also increased significantly following weight loss. The percent increase in OC was positively associated with the magnitude of the weight loss (r=0.25, p=0.02), while the increase in

Study	N	Weight Loss Intervention	Timepoint	Age (y)	Body weight (kg)	BMI (kg/m^2)	Body Fat (%)	VO_{2peak} (ml/kg/min)
[8]	24	-19% (12 weeks)	Baseline	50.1 ± 1.4	103.2 ± 2.2	38.1 ± 0.7	NA	NA
			Weight Loss		83.5 ± 2.0*	30.8 ± 4.5*	NA	NA
[15]	17	-10% (24 weeks)	Baseline	40.0 ± 1.6	94.1 ± 2.6	33.6 ± 0.9	39.4 ± 1.1	23.4 ± 3.0
			Weight Loss		85.5 ± 2.4*	30.6 ± 3.8*	36.2 ± 1.1*	27.5 ± 1.6
[8]	36	-5% (6 weeks)	Baseline	22.9 ± 0.8	75.9 ± 1.5	28.4 ± 0.4	37.4 ± 0.6	27.2 ± 0.8
			Weight Loss		72.1 ± 1.4*	26.9 ± 0.4*	35.5 ± 0.6*	29.1 ± 0.8
[14]	46	Lean, active controls		23.3 ± 1.0	60.2 ± 1.6	21.6 ± 0.5	21.5 ± 0.7	47.2 ± 13.5 (n=6)

Data are means ± SEM. NA, not assessed. BMI, body mass index. *, significantly different from Baseline mean within each study, p<0.05

Table1: Anthropometric characteristics of sedentary, overweight or obese women before and after weight loss compared with active, lean controls.

Study	N	Timepoint	Glucose (mg/dL)	Insulin (pmoL/L)	HOMA2-IR	HOMA2-%B	HOMA2-%S
[8]	24	Baseline	100 ± 3†	88 ± 9†	1.68 ± 0.18†	110 ± 11	80 ± 27†
		Weight Loss	92 ± 2*†	NA	NA	NA	NA
[15]	17	Baseline	93 ± 4†	88 ± 11†	1.65 ± 0.21†	125 ± 13	79 ± 32†
		Weight Loss	81 ± 3*	82 ± 13*†	1.47 ± 0.20†	158 ± 19*	97 ± 30
[8]	36	Baseline	97 ± 3†	65 ± 9†	1.28 ± 0.23†	86 ± 11	143 ± 27†
		Weight Loss	87 ± 2*†	61 ± 11*†	1.11 ± 0.20†	115 ± 17	192 ± 26*
[14]	46	Lean, active controls	82 ± 2	46 ± 7	0.84 ± 0.13	107 ± 8	201 ± 19

Data are means ± SEM. NA, not assessed. HOMA2-IR, -%B, and -%S homeostasis model assessment of insulin resistance, β-cell function, and insulin sensitivity respectively. *,significantly different from Baseline mean within each study; †, significantly different from lean, active controls, P<0.05.

Table2: Plasma glucose, insulin, and homeostasis model estimates of insulin resistance, β-cell function, and insulin sensitivity in sedentary, overweight or obese women before and after weight loss compared with active, lean controls.

Study	N	Timepoint	OC (ng/mL)	BAP (U/L)	CTX (ng/mL)
[8]	24	Baseline	5.4 ± 0.8†	NA	0.699 ± 0.027
		Weight Loss	6.7 ± 0.9*†	NA	0.919 ± 0.036*
[15]	17	Baseline	6.6 ± 1.0†	13.3 ± 2.0†	0.288 ± 0.032
		Weight Loss	8.3 ± 1.0*†	12.6 ± 2.0†	0.327 ± 0.039*
[8]	36	Baseline	11.4 ± 0.9†	17.4 ± 0.7†	0.607 ± 0.044
		Weight Loss	12.5 ± 1.0*	17.6 ± 0.7†	0.700 ± 0.057*
[14]	46	Lean, active controls	14.4 ± 0.6	24.2 ± 1.2	NA

Data are means ± SEM. NA, not assessed. BMI, body mass index; OC, osteocalcin; BAP, bone-specific alkaline phosphatase; CTX, C-terminal peptide of type I collagen*, significantly different from Baseline mean within each study; †, significantly different from lean, active controls, P<0.05

Table3: Serum markers of bone formation and resorption in sedentary, overweight or obese women before and after weight loss compared with active, lean controls

CTX was not (r=0.10, p=0.26). By contrast, serum BAP concentrations were not altered by weight reduction (Table 3).

At baseline, serum OC concentrations were positively correlated with HOMA2-%S (r=0.23, p=0.015) and negatively associated with HOMA2-IR (r=-0.27, p=0.005). Following weight loss, serum OC was negatively associated with fasting glucose (r= -0.232, p=0.02). Weight-loss-associated changes in serum OC were positively correlated with changes in HOMA2-%B (r=0.33, p=0.04). There were no other significant relationships between the bone turnover markers and measures of glucose homeostasis (data not shown).

Discussion

We and others have previously noted that OC increases following weight loss in overweight or obese, sedentary individuals [7-9,18]. In this secondary analysis of data collected from three independent weight-loss interventions [8,9,15], we explored the possibility that the weight-loss-associated increase in OC is related to its potential function as a regulator of glucose homeostasis, rather than its role as a marker of bone formation. We observed that the serum bone formation markers OC and BAP were reduced in overweight or obese women compared with lean, active controls, which is consistent with previous reports of reduced bone formation in association with excess adiposity [19].

However, following weight loss with concurrent reductions in plasma glucose and insulin, only OC increased while BAP remained unchanged. Under conditions of elevated bone formation, such as that induced by pharmacologic treatment [20] or fracture [21], both OC and BAP increase. In addition, after weight reduction, there was an inverse relationship between serum OC and fasting glucose concentration, and weight-loss-associated increases in OC were associated with improved β-cell function estimated using HOMA2. Thus, the post-weight-reduction increase in OC, with no change in BAP, might be related not to bone formation, but to the role of OC as a regulator of glucose homeostasis. Although not confirmatory, these observations are consistent with previously reported cross-sectional associations between OC and measures of insulin sensitivity in humans [18,22-26].

Data from transgenic rodent models indicate that OC regulates glucose homeostasis by both increasing pancreatic secretion of insulin and sensitizing target tissues to insulin action [4,13]. Although one might expect that changes in OC would be correlated with changes in insulin sensitivity, we and others have reported no relationship between changes in OC and fasting glucose and insulin after weight loss [8,18]. However, fasting glucose and insulin do not reflect the capacity of the pancreas to secrete insulin or the sensitivity of muscle to insulin, but rather are measures of hepatic insulin sensitivity [27]. Thus, future studies that assess insulin secretion and sensitivity in response to a glucose load are likely required to accurately evaluate the effects of OC on its target tissues. Moreover, the very recent discovery that human adipose tissue secretes OC *ex vivo* [28], suggests that future studies should consider the source of OC that acts on target tissues to enhance insulin sensitivity.

In addition to an increase in OC, CTX also consistently increased following weight loss (Table 3). This observation and the lack of change in BAP are consistent with the hypothesis that bone mass and strength are governed by a mechanostat [29]. Based on this paradigm, the reduction in mechanical loading on the skeleton following weight loss causes a loss of bone mass that is mediated by a greater increase in bone resorption relative to formation. Additional studies, as described above, are required to determine if the increase in OC following weight loss reflects a change in bone formation or a glucoregulatory response or both.

A limitation of the present study is that we measured total OC, i.e., the sum or carboxylated and uncarboxylated OC. Although in mice it appears that only the uncarboxylated form of OC regulates energy metabolism [4,13], in humans, the roles of the two forms of OC remain unclear [26]. Reported that both total OC and carboxylated OC were associated with fasting glucose and insulin resistance. A recent study in middle-aged men reported that both forms of OC were associated with glucose tolerance, while uncarboxylated OC was related to β-cell function (HOMA-B%) and carboxylated OC was related to insulin sensitivity (HOMA-IR) [23], while Foresta et al reported an inverse association between the uncarboxylated-to-carboxylated OC ratio and BMI in men [28]. Thus, it is possible that we might have observed significant relationships between changes in measures of insulin sensitivity and OC had we differentiated between carboxylated and uncarboxylated OC.

Strengths of the present study include a relatively large sample size and replication of the increase in OC concurrent with improvements in fasting glucose, insulin and HOMA-IR in three independent weight-loss studies. Another strength of the present investigation is use of an anti-OC antibody that recognizes only intact OC, which allowed us to measure OC recently secreted from osteoblasts and not OC fragments released during bone resorption. Thus, although there was an apparent increase in bone resorption following weight loss based on the increase in serum CTX (Table 3), the observed increase in OC was not due to resorptive fragments.

In summary, the results of this preliminary investigation suggest that OC might play a role in the improvements in insulin sensitivity observed following weight reduction in overweight, sedentary individuals. Future studies are needed to substantiate this potential link between bone metabolism and insulin resistance in humans.

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Conflict of Interest

The authors have no competing financial interests in relation to the work described.

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