The Relationship of Serum Osteocalcin Concentration to Insulin Secretion, Sensitivity, and Disposal with Hypocaloric Diet and Resistance Training

Jose Manuel Fernández-Real, Mikel Izquierdo, Francisco Ortega, Esteban Gorostiaga, Javier Gómez-Ambrosi, Jose Maria Moreno-Navarrete, Gema Frühbeck, Cristina Martínez, Fernando Idoate, Javier Salvador, Lluis Forga, Wifredo Ricart, and Javier Ibañez

Department of Diabetes, Endocrinology, and Nutrition (J.M.F.-R., F.O., J.M.M.-N., W.R.). Institut d'Investigació Biomédica de Girona, CIBER Fisiopatología de la Obesidad y Nutrición CB06/03/010, 17007 Girona, Catalonia, Spain; Studies, Research, and Sports Medicine Center (M.I., E.G., C.M., J.I.), Government of Navarra, 31005 Pamplona, Spain; Department of Endocrinology (J.G.-A., G.F., J.S.), University of Navarra, and CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, 31008 Pamplona, Spain; Department of Radiology (F.I.), Clinic of San Miguel, Pamplona-Navarra, Spain; and Department of Endocrinology (L.F.), Hospital of Navarra, 31005 Pamplona-Navarra, Spain

Context: Bone has recently been described as exhibiting properties of an endocrine organ by producing osteocalcin that increases insulin sensitivity and secretion in animal models.

Objective and Design: We aimed to evaluate circulating osteocalcin in association with insulin sensitivity and insulin secretion in three different studies in nondiabetic subjects: one cross-sectional study in 149 men (using minimal model), and two longitudinal studies in two independent groups (one formed by 26 women, and the other by 9 men and 11 women), after a mean of 7.3 and 16.8% weight loss, and after a mean of 8.7% weight loss plus regular exercise.

Results: In the cross-sectional study, circulating osteocalcin was associated with insulin sensitivity, mainly in lean subjects, and with insulin secretion (only in lean subjects). A mean of 16.8%, but not 7.3% weight loss, led to significant increases in circulating osteocalcin. However, a mean of 8.7% weight loss plus regular exercise led to the more pronounced effects on the serum osteocalcin concentration, which increased in parallel to reduced visceral fat mass, unchanged thigh muscle mass, and increased leg strength and force. The postintervention serum levels of osteocalcin were associated with both insulin sensitivity ($r = 0.49$; $P = 0.03$) and fasting triglycerides ($r = -0.54$; $P = 0.01$). The change in visceral fat was the parameter that best predicted the change in serum osteocalcin, once age, body mass index, and insulin sensitivity changes were controlled for ($P = 0.002$).

Conclusion: Circulating osteocalcin could mediate the role of bone as an endocrine organ in humans. (***J Clin Endocrinol Metab* 94: 237–245, 2009**)

Abnormalities of bone metabolism are well known to occur in subjects with obesity and type 2 diabetes. Even increased adiposity in children is a risk factor for fracture (1). Patients with type 2 diabetes are prone to fracture, although their bone density may not be particularly low (2). The rate of bone turnover is decreased in patients with type 2 diabetes, as reflected by diminished expression of biomarkers of bone resorption and formation, including osteocalcin, an osteoblast-specific protein (3). In fact, several studies have previously demonstrated that serum osteocalcin was reduced in patients with type 2 diabetes (4–7).

Lee *et al.* (8) have recently demonstrated in mice that bone regulates the insulin/glucose axis and energy metabolism. This is a fascinating new concept according to which the bone behaves as an endocrine organ by secreting osteocalcin, which leads to increased insulin secretion, lower blood glucose, increased insulin sensitivity, decreased visceral fat, and increased energy ex-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2008-0270 Received February 4, 2008. Accepted October 7, 2008.
First Published Online October 14, 2008

Abbreviations: BMI, Body mass index; C, control group; CV, coefficient of variation; D, diet group; HOMA, homeostatic model assessment; MR, magnetic resonance; RT, resistance training.

penditure. In fact, mice lacking osteocalcin displayed decreased β-cell proliferation and insulin resistance, an abnormal amount of visceral fat, and increased serum triglyceride levels (8). Similar information in humans is lacking.

This recent description of osteocalcin as a bone-derived hormone impacting on insulin sensitivity in animal models provided us a framework to test whether circulating osteocalcin could also be associated with metabolic effects in humans. In fact, there are few studies that have evaluated circulating osteocalcin in relation to insulin sensitivity in humans.

Subjects and Methods

Cross- sectional study

A total of 149 consecutive men [mean age, 50.2 ± 11.7 yr; range, 30–68 yr; mean body mass index (BMI) 27.6 ± 3.5 kg/m^2] were recruited in an ongoing study dealing with insulin sensitivity in northern Spain. Subjects were randomly located from a census, and they were invited to participate. Participation rate was 71%. Inclusion criteria were: 1) BMI < 40 kg/m^2; 2) absence of systemic disease; and 3) absence of infection within the previous month. None of the control subjects were taking medications or had evidence of metabolic diseases other than obesity. Liver disease and thyroid dysfunction were specifically excluded by biochemical work-up. All subjects had fasting plasma glucose below 7.0 mM and were taking no medications. Type 2 diabetes was ruled out by an oral glucose tolerance test according to criteria from the American Diabetes Association. Insulin sensitivity was measured using the frequently sampled iv glucose tolerance test with minimal model analysis. Insulin secretion was calculated as the insulin area during the first 10 min of the frequently sampled iv glucose tolerance test. This test also provides the insulin disposition index, a parameter emerging from the model, which represents the ability of the pancreatic islets to compensate for insulin resistance.

In brief, the experimental protocol started between 0800 and 0830 h after an overnight fast. A needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10, and −5 min, after which glucose (300 mg/kg body weight) was injected over 1 min starting at time 0, and insulin (Actrapid, 0.03 U/kg; Novo Nordisk, Bagsvaerd, Denmark) was administered at time 20 min. Additional samples were obtained from a contralateral antecubital vein up to 180 min.

Effects of slight weight loss with or without regular physical activity

Sedentary, nonsmoking, obese (BMI, 30–40 kg/m^2) women, aged 40–60 yr, were recruited through an advertisement in a local newspaper. Before inclusion in the study, all candidates were thoroughly screened by an extensive medical history, resting and maximal exercise electrocardiograms, and blood pressure measurements. Cardiovascular, neuromuscular, arthritic, pulmonary, or other debilitating diseases as determined via one or all of the screening tools were reasons for exclusion from the study. None of the subjects received any medication. All subjects were carefully informed about the possible risks and benefits of the project and then provided written consent forms before participating in the study. This project was approved by the ethical committee of the regional health department.

Participants were randomized to three groups: a control group (C; n = 7); a diet group (D; n = 8) with a caloric restriction of 500 kcal/d; and a diet and resistance training group (D+RT; n = 11) with the same caloric restriction as group D and a 16-wk supervised RT program of two sessions per week. During the 16 wk of the study, the subjects maintained their customary recreational physical activities (*e.g.* walking). The baseline characteristics of the subjects are presented in Table 1.

TABLE 1. Baseline and follow-up characteristics of the slight weight loss study

	Control (n = 7), age 51.5 ± 7.2 yr			Diet (n = 8), age 51.6 ± 6.6 yr			Diet + exercise (n = 11), age 47.7 ± 6.5 yr			P^a
	Pre	Post	*P*	Pre	Post	*P*	Pre	Post	*P*	*(age, 0.36)*
BMI (kg/m^2)	34.58 ± 3.90	34.38 ± 3.55	0.52	34.11 ± 3.89	31.62 ± 3.92	0.009	34.27 ± 2.78	31.33 ± 2.00	<0.0001	0.96
Weight (kg)	88.68 ± 12.74	88.15 ± 11.74	0.506	87.22 ± 18.01	80.81 ± 16.64	0.012	88.08 ± 12.63	80.36 ± 9.11	<0.0001	0.98
WHR	0.91 ± 0.01	0.89 ± 0.02	0.26	0.93 ± 0.02	0.91 ± 0.03	0.08	0.92 ± 0.03	0.88 ± 0.03	0.026	0.126
Thigh muscle area	46,667.6 ± 7,736	46,465.5 ± 7,167.4	0.74	48,966.4 ± 10,883.2	47,226.8 ± 11,609	0.039	46,867.6 ± 9,021.3	46,470.2 ± 8,839.2	0.87	0.87
Thigh fat area	88,332 ± 19,339	89,127.2 ± 19,090.9	0.59	87,641.9 ± 21,716.5	72,697.6 ± 18,365.1	0.004	104,923.7 ± 16,539.6	87,479.1 ± 14,200.8	<0.0001	0.11
Visceral AT	3,370.78 ± 1,228.42	3,329.26 ± 1,187.23	0.65	3,243.75 ± 1,085.33	2,557.51 ± 1,171.16	0.007	3,211.30 ± 1,232.09	2528.43 ± 1039.89	0.0001	0.96
Total AT	16,833.4 ± 4,183.2	16,623.5 ± 3,785.1	0.53	16,973.7 ± 4,787.1	13,333.2 ± 4,840.8	0.001	18,205 ± 3,337.9	13810.7 ± 2201.8	<0.0001	0.73
1RM arms	34.28 ± 6.56	34.81 ± 6.38	0.20	30.31 ± 5.25	29.21 ± 5.86	0.175	32.95 ± 6.96	43.63 ± 7.61	<0.0001	0.47
1RM legs	187.28 ± 30.47	188.71 ± 31.25	0.17	182.87 ± 39.80	205.37 ± 67.07	0.09	175.00 ± 33.68	274.54 ± 64.00	<0.0001	0.75
HOMA	4.00 ± 1.54	3.01 ± 1.50	0.08	3.93 ± 2.61	2.92 ± 2.20	0.066	3.19 ± 1.53	2.13 ± 1.07	0.029	0.605
Adiponectin	13.17 ± 3.24	12.67 ± 2.19	0.44	12.33 ± 4.85	12.68 ± 4.24	0.63	14.15 ± 4.55	12.90 ± 3.55	0.106	0.66
Log sTNFR2	0.76 ± 0.03	0.75 ± 0.05	0.97	0.74 ± 0.10	0.69 ± 0.05	0.15	0.73 ± 0.15	0.75 ± 0.11	0.65	0.98
Log osteocalcin	0.24 ± 0.35	0.07 ± 0.56	0.12	0.32 ± 0.31	0.31 ± 0.43	0.87	0.02 ± 0.39	0.34 ± 0.19	0.006	0.63

Values are mean ± SD. AT, Adipose tissue; 1RM, one repetition maximum; sTNFR2, serum TNF receptor 2; WHR, waist-to-hip ratio.

[a] ANOVA *P* for baseline characteristics among groups.

Diet

Diet was designed, in both D and D+RT groups, to reduce 500 kcal/d according to a previous evaluation of the habitual physical activity of each subject by accelerometry (TriTrac-R3D System, Software Version 2.04; Reining International, Madison, WI). This diet was designed to elicit a 0.5-kg weight loss per week. The C group was asked to maintain body weight. Throughout the 16-wk intervention period, body weight was recorded every 2 wk in both D and D+RT groups. Each subject of the intervention groups participated in a series of 1-h seminars (every 2 wk) wherein the dietitian taught proper food selection and preparation, eating behavior, control of portion sizes, and modification of binge eating and other adverse habits. The average compliance with the diet classes and the exercise sessions was above 95%.

RT program

The strength training program was a combination of heavy resistance and "explosive" strength training. The subjects were asked to report to the training facility two times per week for 16 wk to perform dynamic resistance exercise for 45–60 min per session. A minimum of 2 d elapsed between two consecutive training sessions. Each training session included two exercises for the leg extensor muscles (bilateral leg press and bilateral knee extension exercises), one exercise for the arm extensor muscle (the bench press), and four to five exercises for the main muscle groups of the body. Only resistance machines (Technogym, Gambettola, Italy) were used throughout the training period. In all the individual exercise sessions performed, one of the researchers was present to direct and assist each subject toward performing the appropriate work rates and loads. Lower and upper body maximal strength was assessed at wk 0 and 16 by using one repetition-maximum actions.

Magnetic resonance (MR)

The volumes of visceral and abdominal sc adipose tissue were measured by MR. MR imaging was performed with a 1T magnet (Magnetom Impact Expert; Siemens Corporation, New York, NY) using body coil. The subjects were examined in a supine position with both arms positioned parallel along the lateral sides of the body. The following procedures, in chronological order, were carried out: upper part of the body, subject repositioning; and lower part acquisition. We obtained a spoiled T1 weighted gradient-echo sequence with repetition time (TR) = 127 msec and echo time (TE) = 6 msec. Each half body volume was scanned using two stacks, each containing 10 contiguous 10-mm-thick slices. Each stack was acquired in 20 sec, and interleaved slice order was used. A field of view of 500 mm was used, and all the stacks were acquired with breath holding. Depending on the height of the person, this resulted in a total of 31–40 axial images per person. The total investigation time was about 5 min.

MR imaging of both thighs was then obtained. T1-weighted sequence was used with a repetition time (TR) of 645 msec and a spin echo time (TE) of 20 msec. The field of view was 500 × 500 mm, and the matrix was 512 × 192. The slices were 10 mm thick, with no gap between the slices. The thighs were scanned using two stacks, each containing 15 contiguous 10-mm-thick slices; the scan was performed axially from articular boundary of lowest external femoral condyle. The images were retrieved from the scanner according to a DICOM (Digital Imaging and Communications in Medicine) protocol. The acquired axial MR images were transferred to an external personal computer running Windows XP. The level of each abdominal image was labeled using sagittal scout images, referred to discal level. We used a specially designed image analysis software (SliceOmatic 4.3, Tomovision Inc., Montreal, Canada) for quantitative analysis of the images.

Effects of moderate weight loss

To evaluate further the effect of moderate weight loss on circulating osteocalcin after weight loss, 20 Caucasian obese volunteers (9 males, 11 females; age range, 21 to 66 yr) attending the Endocrinology Department at the University Clinic of Navarra were recruited. Patients underwent a clinical assessment including medical history, physical examination, body composition analysis, and comorbidity evaluation, as well as nutritional interviews performed by a multidisciplinary consultation team. All subjects were nonsmokers. Patients with signs of infection were excluded. Obese patients were not receiving statins or any antidiabetic medication.

Type 2 diabetes mellitus was defined following the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus based on both fasting plasma glucose concentrations and plasma glucose 2 h after an oral glucose tolerance test.

Diet

Weight loss was achieved by prescription of a diet providing a daily energy deficit of 500–1000 kcal/d as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29; SensorMedics Corporation, Yorba Linda, CA) and multiplication by 1.4 as indicated for sedentary individuals to obtain the patient's total energy expenditure (9). This hypocaloric regime allows a safe and steady weight loss of 0.5–1.0 kg/wk when strictly followed and supplied 30, 54, and 16% of energy requirements in the form of fat, carbohydrates, and protein, respectively.

In this study, body weight was measured with a digital scale to the nearest 0.1 kg, height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain Ltd., Crymych, UK), and body fat was estimated by air-displacement-plethysmography (Bod-Pod; Life Measurements, Concord, CA). Data for estimation of body fat by this plethysmographic method has been reported to agree closely with the traditional gold standard hydrodensitometry (underwater weighing) (10).

The experimental design was approved, from an ethical and scientific standpoint, by the Hospital's Ethical Committees from all participant institutions in the three different studies, and volunteers gave their informed consent to participate in all the studies.

Analytical determinations

In all studies, blood samples were collected after an overnight fast in the morning to avoid potential confounding influences due to hormonal rhythmicity. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Intraassay and interassay coefficients of variation (CVs) were less than 4% for all these tests.

Measurements of serum adiponectin and plasma osteocalcin were centralized in a single laboratory. Osteocalcin was measured by an Enzyme Amplified Sensitivity Immunoassay (EASIA) kit (DRG Instruments GmbH, Marburg, Germany). Sensitivity of the method, the detection limit, defined as the apparent concentration two SD values above the average OD at zero binding, was 0.4 ng/ml, and the intra- and interassay CVs were less than 10%. Serum adiponectin levels were measured by a commercially available ELISA kit (Linco Research, St. Charles, MO). The intra- and interassay CVs were less than 8%. The lowest level of adiponectin that can be detected by this assay is 0.78 ng/ml. There was no cross-reactivity with other cytokines or hormones.

In the cross-sectional study, serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Serum insulin levels were measured in duplicate by monoclonal immunoradiometric assay [IRMA or enzyme-amplified sensitivity immunoassay (EASIA), Medgenix Diagnostics, Fleunes, Belgium]. Intraassay and interassay CVs were similar to those previously reported (11, 12).

In the study of the effects of slight weight loss with or without regular physical activity, resting blood samples were drawn at wk 0 and 16. The subjects reported to the laboratory and sat quietly for 10–15 min before giving a blood sample. Basal glycemia was analyzed using an enzymatic hexokinase method (Roche Diagnostics, Mannheim, Germany). Serum insulin levels were measured in duplicate by monoclonal immunoradiometric assay (INSI-CTK Irma; DiaSorin, Madrid, Spain). Intraassay and interassay CVs were less than 5%. To estimate insulin resistance, the homeostatic model assessment (HOMA) index was calculated as fast-

ing insulin concentration (μU/ml) × fasting glucose concentration (mmol/liter)/22.5.

In the study of the effects of moderate weight loss, plasma glucose was analyzed by an automated analyzer (Roche/Hitachi Modular P800, Basel, Switzerland) as previously described (13). Insulin was measured by means of an enzyme-amplified chemiluminescence assay (Immulite, Diagnostic Products Corp., Los Angeles, CA). An indirect measure of insulin sensitivity was calculated from the fasting plasma glucose and insulin concentrations by using the quantitative insulin sensitivity check index (14, 15).

Statistical analysis

Pearson's correlation was used to evaluate the associations among continuous variables. Those parameters that did not follow a normal distribution were log-transformed. Comparisons of quantitative variables among groups were made using ANOVA. Multiple regression models were used to assess the influence of osteocalcin on insulin sensitivity, taking into account potential factors associated with this variable such as BMI and waist-to-hip ratio. The models were built in a customized way by means of the enter method, which takes into account the simultaneous influence of all variables; this is a procedure for variable selection in which all variables in a block are entered in a single step. We chose this conservative method given the relatively low number of subjects studied. Furthermore, regression diagnostics were checked by using the inverse normal plot of the residuals and plots of the residuals against the fitted values. Influence analyses were also performed by means of Cook's D. Moreover, the problems of colinearity were solved by centering some of the variables. The statistical package used was Stata v.8 (StataCorp, College Station, Texas). Levels of statistical significance were set at $P < 0.05$.

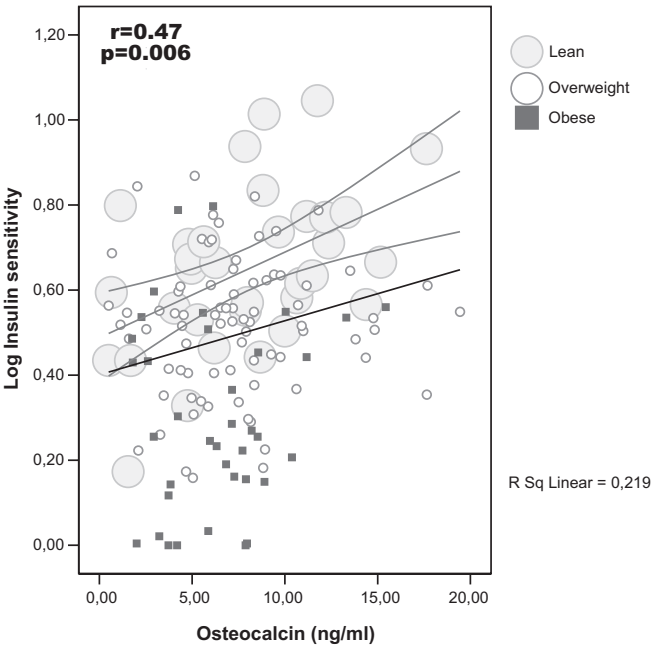

FIG. 1. Linear relationship between circulating osteocalcin and insulin sensitivity in 149 men of the cross-sectional study (single line and correlation coefficient) and in lean men (95% confidence interval for the mean and correlation coefficient in the *upper left corner*). Lean subjects are defined as having a BMI less than 25 kg/m^2; overweight as BMI at least 25 but less than 30 kg/m^2; and obese as BMI greater than 30 kg/m^2. We used the nontransformed value of osteocalcin because the Kurtosis and skewness values were closer to 0 than the log-transformed value in this population.

Results

Cross-sectional study

We evaluated 149 men, aged 50.2 ± 11.7 yr, with mean BMI 27.6 ± 3.5 kg/m^2, a median insulin sensitivity of $2.35*10^{-4}*min^{-1}*$mU/liter (interquartile range, 1.23–3.2), and a median insulin secretion of 359.1 mU/liter*min^{-1} (interquartile range, 186.6–511.1). Median circulating osteocalcin was 6.1 (interquartile range, 3.5–8.1) ng/ml. Osteocalcin was positively linked to insulin sensitivity among these 149 otherwise healthy men (r = 0.23; $P = 0.006$; Fig. 1). The statistical power of this association was 81% ($\alpha = 0.05$, $\beta = 0.20$).

Interestingly, the association appeared stronger in lean subjects (BMI < 25 kg/m^2) (Fig. 1, *enclosed legend*), although the comparison between slopes did not reach statistical significance ($P = 0.3$). Among lean subjects, osteocalcin was the most significant factor impacting on insulin sensitivity (45% of its variance), even after accounting for the effects of age, BMI, and waist diameter in a multiple linear regression analysis. Among lean subjects, we also observed a positive association between circulating osteocalcin and insulin secretion (r = 0.41; $P = 0.03$) and the insulin disposition index (r = 0.43; $P = 0.02$). Circulating adiponectin was available in 137 of these subjects and showed a positive association with osteocalcin (r = 0.19; $P = 0.02$).

Effects of weight loss on circulating osteocalcin

Given this cross-sectional association, we also aimed to evaluate the effects of weight loss and physical exercise on circulating osteocalcin. Twenty-six obese women were randomized to follow a structured RT program and a hypocaloric diet (D+RT; n = 11), compared with only a hypocaloric diet (D; n = 8) and a control group (C; n = 7), in which no action was taken. Baseline characteristics were similar in the three groups (Table 1). Serum osteocalcin was negatively associated with insulin resistance (HOMA value, r = −0.43; $P = 0.03$) and positively with circulating adiponectin (r = 0.45; $P = 0.02$). Baseline osteocalcin was not significantly associated with total fat (r = −0.27; $P = 0.18$), visceral fat (r = −0.24; $P = 0.2$), or fasting triglycerides (r = 0.01; $P = 0.9$).

After 16 wk, no significant changes were observed in the different parameters evaluated in the control group (Table 1). In the diet group, a 7.3% weight loss was accompanied by reduced total and visceral fat mass and thigh muscle mass. Insulin sensitivity tended to improve. No significant changes in serum osteocalcin concentrations were observed. In the diet plus RT group, despite the fact that weight loss was of similar magnitude (−8.7%), osteocalcin increased significantly (Fig. 2). The statistical power of this change in serum osteocalcin was 96% ($\alpha = 0.05$, $\beta = 0.20$). This was observed in parallel with reduced visceral fat mass, unchanged thigh muscle mass, and increased leg strength and force (Fig. 3). In all subjects as a whole (n = 26), the change in circulating osteocalcin was significantly associated with the change in visceral fat (r = −0.59; $P = 0.001$). However, in the subgroup analysis, this relationship was not significant in the control group (r = 0.25; $P = 0.6$) or in the diet group (r = −0.40; $P = 0.3$).

In the intervention groups (both D and D+RT, n = 19) the postintervention serum levels of osteocalcin were associated

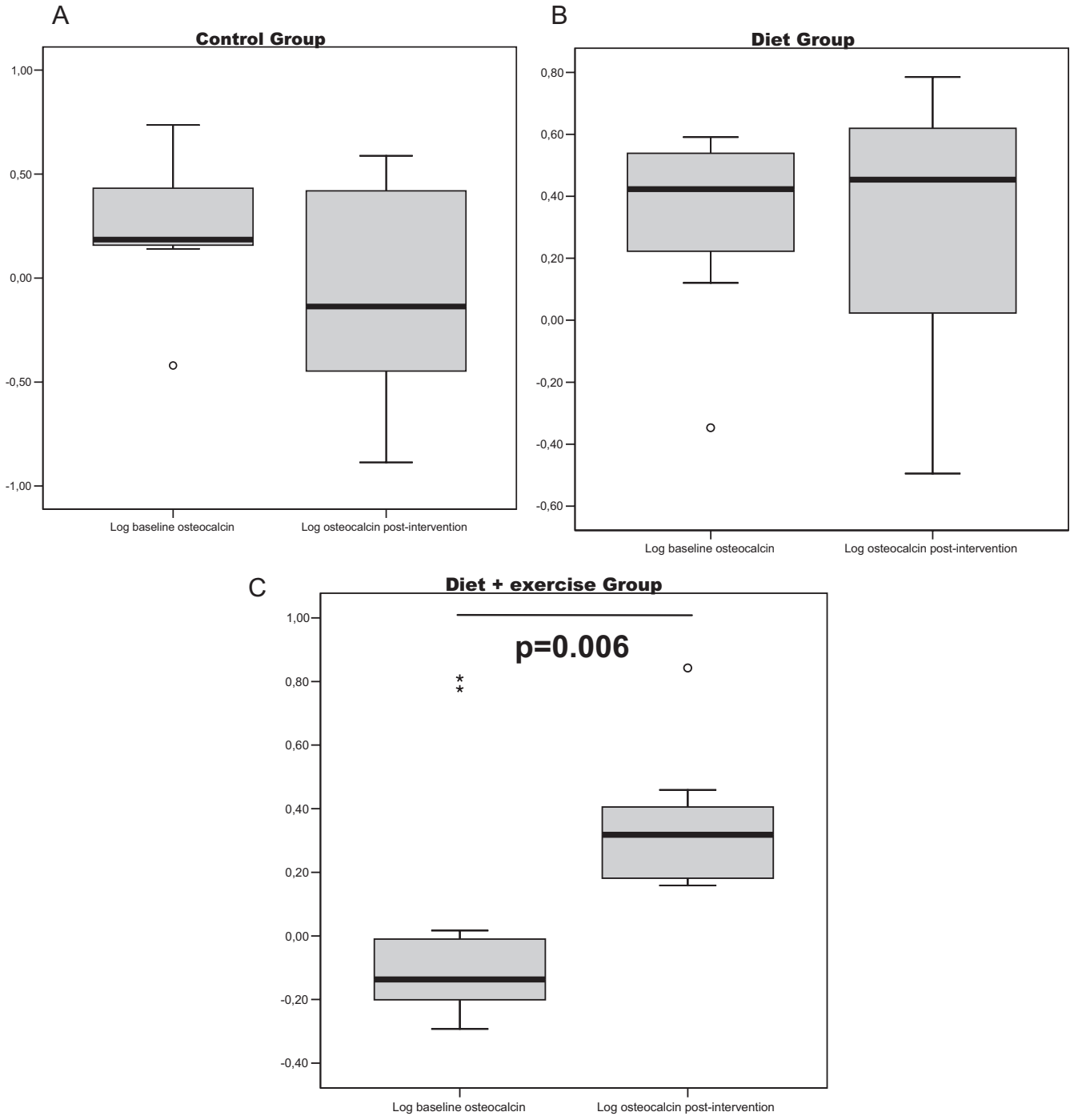

FIG. 2. Changes in circulating osteocalcin in obese women (n = 26) enrolled in the slight weight loss intervention. A, Control subjects, in whom no action was taken (n = 7); B, diet-induced weight loss (n = 8); C, weight loss induced by diet plus regular exercise (n = 11). *Edges of gray box* indicate 25th and 75th percentiles. *Horizontal line in middle of box* depicts median. *Whiskers* indicate minimum/maximum values.

with both insulin resistance ($r = -0.49$; $P = 0.03$) and fasting triglycerides (Fig. 3D). However, we did not observe significant relationships between the change in circulating osteocalcin and the change in fasting triglycerides. In all subjects as a whole (n = 26), the change in visceral fat was the single parameter that best predicted the change in serum osteocalcin, once age, BMI, and insulin sensitivity changes were controlled for ($P = 0.002$) (Table 2). When the change in leg muscle strength was introduced in the model, both variables contributed to 30% of the variance in changing serum osteocalcin (Table 2).

We then questioned whether the magnitude of weight loss was insufficient to impact on circulating osteocalcin concentration. To this end, we studied subjects that underwent a more prolonged period of treatment, achieving a mean weight loss of −16.8%. The characteristics of these subjects are shown in Table 3. Baseline osteocalcin was not significantly associated with insulin sensitivity ($r = 0.25$, $P = 0.3$) and tended to be negatively associated with total fat mass ($r = -0.33$, $P = 0.1$). In these subjects, mean osteocalcin was increased after weight loss (Fig. 4). The statistical power of this change in serum osteocalcin was 78%

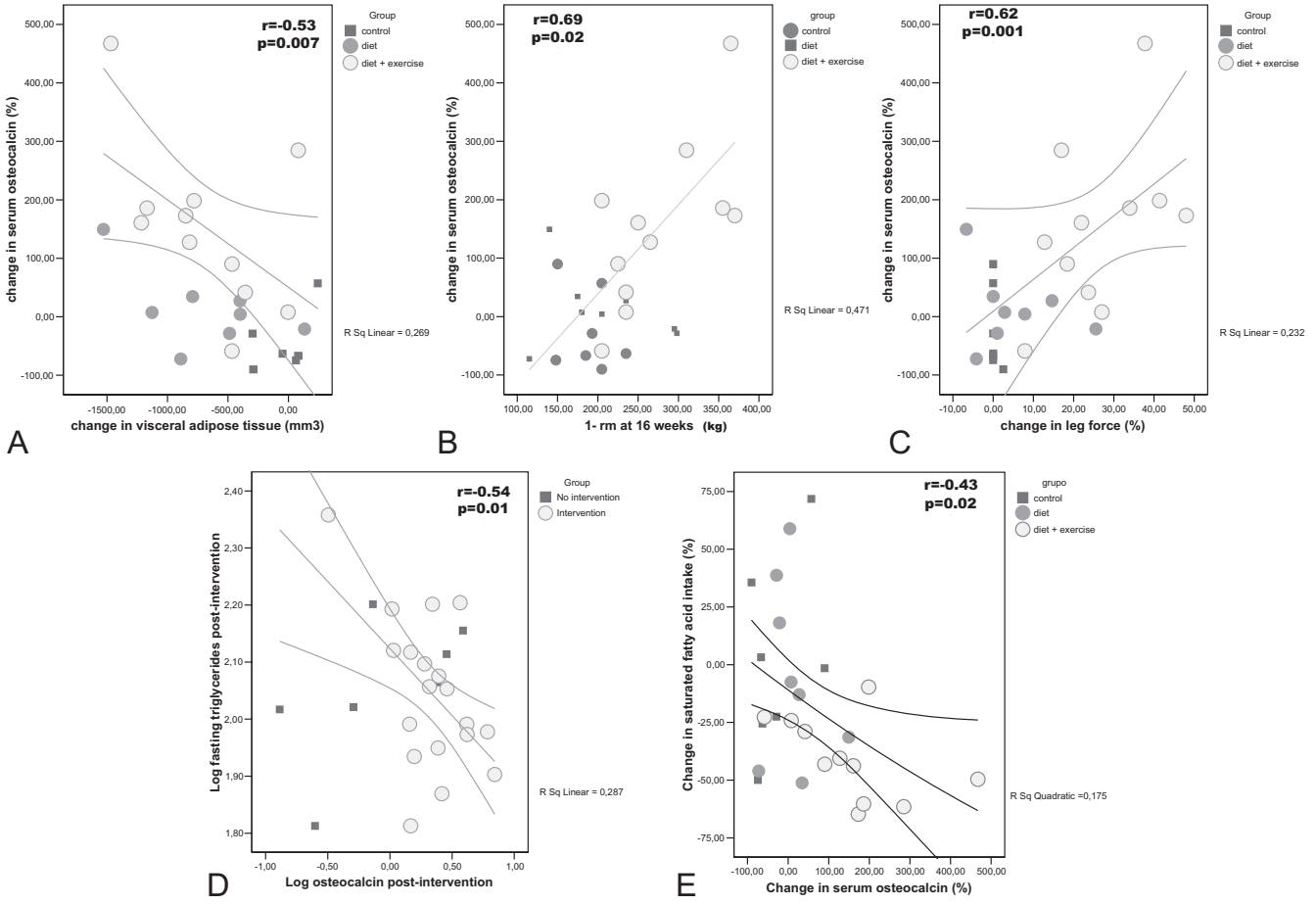

FIG. 3. Factors associated with changing osteocalcin in obese women (n = 26) enrolled in the slight weight loss intervention. Variables associated with the change in serum osteocalcin: A, change in visceral adipose tissue; B, absolute leg force (1-rm); and C, changes in leg force. D, Log osteocalcin was associated with fasting triglycerides only after weight loss in the intervention group as a whole: diet only and diet + RT groups together. E, Relationship between the change in saturated fatty acid intake and change in osteocalcin after weight loss. The coefficients shown only represent *open circles* in all panels.

($\alpha = 0.05$, $\beta = 0.20$). However, we found no associations between the change in serum osteocalcin and changing insulin resistance, circulating adiponectin, or triglycerides (r values between −0.32 and 0.26, $P > 0.1$). Interestingly, among men, the decrease in waist diameter tended to be associated with the increase in osteocalcin but this was not statistically significant (r = −0.51, $P = 0.1$, n = 9).

Discussion

Summarizing the associations with insulin sensitivity, we found that fasting osteocalcin was associated with insulin sensitivity cross-sectionally in 149 men and in 26 obese women with a wide range of BMI, but not in 20 obese men and women with a low BMI range. The change in circulating osteocalcin was significantly associated with the change in insulin sensitivity in the slight weight loss group (both D and D+RT groups, r = −0.50; $P = 0.02$; n = 19) but not in the moderate weight loss group.

TABLE 2. Multiple linear regression analysis with the change in circulating osteocalcin as dependent variable in the slight weight loss study

Change in osteocalcin	β	*P* value	β	*P* value
Age	−0.17	0.34	−0.009	0.85
Change in BMI	−0.12	0.57	−0.19	0.87
Change in insulin sensitivity	−0.10	0.58	−0.12	0.94
Change in visceral fat	−0.52	0.002	−0.46	0.005
Change in leg muscle strength			0.51	0.002
Adjusted R^2	0.24		0.30	

TABLE 3. Effect of moderate weight loss in obese patients after a dietary intervention

n	Before weight loss 20	After weight loss 20	*P*
Age (yr)	43.4 ± 9.4	44.1 ± 9.2	
Body weight (kg)	109 ± 7	91 ± 4	<0.001
BMI (kg/m^2)	38.0 ± 2.0	32.0 ± 1.2	<0.0001
Body fat (%)	45.9 ± 2.0	38.6 ± 2.1	<0.0001
Waist circumference (cm)	115 ± 4	103 ± 3	<0.0001
WHR	0.95 ± 0.02	0.94 ± 0.02	0.175
Glucose (mmol/liter)	5.6 ± 0.2	5.1 ± 0.1	0.043
Insulin (mU/ml)	21.4 ± 5.2	11.8 ± 1.7	0.112
QUICKI	0.312 ± 0.009	0.341 ± 0.012	0.031
Leptin (ng/ml)	39.2 ± 12.1	25.4 ± 10.2	0.044

To convert glucose to milligrams per deciliter, divide by 0.05551. WHR, Waist-to-hip ratio.

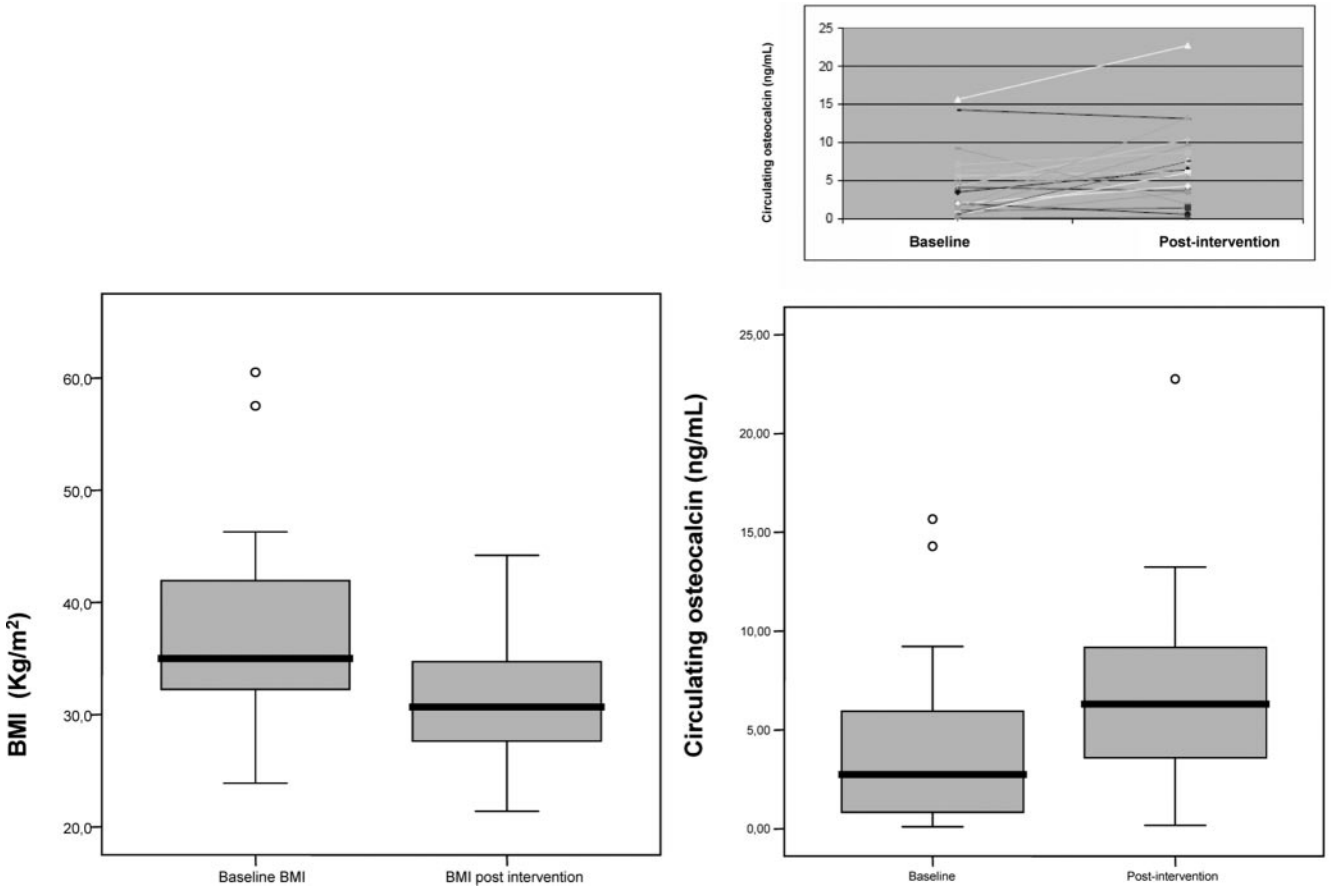

FIG. 4. Obese men and women (n = 20) enrolled in the moderate weight loss intervention. Changes in BMI (*left panel*) and osteocalcin (*right panels*). *Edges of gray box* indicate 25th and 75th percentiles. *Horizontal line in middle of box* depicts median. *Whiskers* indicate minimum/maximum values.

The main findings of this study are: 1) the association between circulating osteocalcin and insulin sensitivity; 2) the association between osteocalcin and insulin secretion and insulin disposition index among lean men; 3) the observation that slight diet-induced weight loss *per se* did not lead to significant changes in serum osteocalcin concentration; 4) a weight loss of similar magnitude plus regular physical activity resulted in increased circulating osteocalcin; 5) the increase in serum osteocalcin concentration was associated with changes in visceral fat mass and, importantly, with changes in leg muscle strength; and 6) moderate weight loss also resulted in increased osteocalcin but without relationship to insulin sensitivity or fasting triglycerides.

In parallel with the findings described in experimental animals (8), we found that the baseline circulating osteocalcin concentration was associated with insulin sensitivity and secretion and circulating adiponectin (lean and obese men and women). After slight weight loss, osteocalcin correlated with fasting triglycerides in obese women.

Osteocalcin knockout mice showed increased visceral fat (8). In our study, serum osteocalcin significantly increased in parallel to reduced visceral fat mass after diet and regular exercise in obese women. In the slight weight loss study, baseline osteocalcin was not significantly different among groups (Table 1). By chance, mean values of osteocalcin were higher in the D+RT group compared with the other groups. This difference was not statistically significant, even if we compared this group to the remaining subjects ($P = 0.3$). In fact, baseline mean log osteocalcin in the D+RT group was very similar to that present in the control group after follow-up.

It could be argued that the change in insulin resistance and fat mass was, to some extent, similar in the D group and the D+RT group (the change in insulin resistance in the D group did not reach statistical significance). However, the most striking differences between these groups were the change in leg force, which was strongly related with the change in serum osteocalcin in univariant and multivariate analysis. Thigh muscle mass was unchanged after diet plus regular exercise in association with increased leg strength and force. In contrast, in the D group, thigh muscle mass was significantly decreased after 16 wk (Table 1). In a previous study, as little as a 5% weight loss plus regular exercise also led to increased osteocalcin (16).

There is a considerable body of evidence gathered from studies over the past half century indicating that regular physical activity reduces the risk of cardiovascular disease. Regular physical activity is particularly beneficial to individuals with insulin-resistant conditions, such as obesity, type 2 diabetes, and the metabolic syndrome (17). Although the postexercise increase in muscle insulin sensitivity has been characterized in considerable detail, the basic mechanisms underlying this phenomenon remain a mystery (18). Like exercise, stimulation of muscles to contract *in situ* results in an increase in insulin sensitivity (19). In contrast, stimulation of muscles immersed in Krebs-Henseleit-

bicarbonate buffer to contract *in vitro* does not result in enhanced insulin sensitivity (18–20). The explanation for this finding was that an as-yet-unidentified serum protein must be present during contractile activity in order for the increase in insulin sensitivity to occur (20). The mechanism responsible for the permissive effect of serum has not yet been elucidated. Also, like contractile activity, the effects of exercise, hypoxia, and 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (AICAR, a pharmacological activator of AMPK) on insulin sensitivity require the presence of serum during the treatment period (21).

We propose that osteocalcin represents this missing link in the exercise-induced improvement in insulin sensitivity. Exercise is thought to act on the skeleton through muscle pull, producing strains on the skeleton that are perceived by bone cells. We observed that a change in leg force was associated with a change in serum osteocalcin concentration (Fig. 3C). Exercise may stimulate increased secretion of osteocalcin by bone that positively impacts on insulin secretion and insulin sensitivity. We further propose that diet-induced weight loss and exercise lead to changes in insulin sensitivity by different mechanisms. Although prolonged dieting induced changes in circulating osteocalcin, the magnitudes of these changes were not associated with the metabolic profile.

Moderate, but not slight, weight loss led to significantly increased circulating osteocalcin levels, possibly indicating only increased bone turnover. This supports previous findings in which osteocalcin increased after diet-induced weight loss (22, 23). As previously suggested, the overall increase in bone turnover may be unfavorable for maintaining bone mass after diet-induced weight loss (22). A study of the ratio of undercarboxylated osteocalcin to total osteocalcin after diet and after exercise might provide the clue for the study of their association with insulin sensitivity.

Not all reports on the effects of weight loss or exercise on circulating osteocalcin levels are concordant. Villareal *et al.* (24) reported no significant changes in osteocalcin levels with weight loss due to caloric restriction. However, no obese subjects were included in this study (24). Interestingly, these authors found that exercise was associated with preservation of bone mineral density that could be mediated through exercise-induced bone loading (24). We here suggest that bone loading could elicit increased osteocalcin production. On the other hand, weight gain also led to increased osteocalcin in patients with anorexia nervosa, possibly indicating, again, increased bone remodeling (25).

Several studies have previously demonstrated that serum osteocalcin was reduced in patients with type 2 diabetes (4–7). To our knowledge, this would be the first study evaluating osteocalcin in association with insulin sensitivity in humans, and the first study showing exercise-induced changes in circulating osteocalcin in association with insulin sensitivity, visceral fat mass, and muscle strength. However, the lack of data on undercarboxylated osteocalcin is a limitation of this study. Lee *et al.* (8) reported that undercarboxylated osteocalcin was the active form of osteocalcin in rodent models.

In summary, our findings suggest that osteocalcin might be an active regulator of insulin sensitivity by bone.

Acknowledgments

Address all correspondence and requests for reprints to: Jose Manuel Fernandez-Real, M.D., Ph.D., Unit of Diabetes, Endocrinology, and Nutrition, Hospital de Girona "Dr. Josep Trueta", Ctra. França s/n, E-17007 Girona, Catalonia, Spain. E-mail: uden.jmfernandezreal@htrueta.scs.es.

Disclosure Statement: The authors have nothing to disclose. No potential conflict of interest relevant to this article is reported.

References

1. **Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ** 2001 Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr 139:509–515
2. **Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, Jamal SA, Black DM, Cummings SR** 2001 Study of osteoporotic features research group. Older women with diabetes have an increased risk of fracture: a prospective study. J Clin Endocrinol Metab 86:32–38
3. **Gerdhem P, Isaksson A, Akesson K, Obrant KJ** 2005 Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus. Osteoporos Int 16:1506–1512
4. **Bouillon R, Bex M, Van Herck E, Laureys J, Dooms L, Lesaffre E, Ravussin E** 1995 Influence of age, sex, and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. J Clin Endocrinol Metab 80:1194–1202
5. **Pietschmann P, Schernthaner G, Woloszczuk W** 1988 Serum osteocalcin levels in diabetes mellitus: analysis of the type of diabetes and microvascular complications. Diabetologia 31:892–895
6. **Pedrazzoni M, Ciotti G, Pioli G, Girasole G, Davoli L, Palummeri E, Passeri M** 1989 Osteocalcin levels in diabetic subjects. Calcif Tissue Int 45:331–336
7. **Rico H, Hernandez ER, Cabranes JA, Gomez-Castresana F** 1989 Suggestion of a deficient osteoblastic function in diabetes mellitus: the possible cause of osteopenia in diabetics. Calcif Tissue Int 45:71–73
8. **Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G** 2007 Endocrine regulation of energy metabolism by the skeleton. Cell 130:456–469
9. **Gómez-Ambrosi J, Catalán V, Ramírez B, Rodríguez A, Colina I, Silva C, Rotellar F, Mugueta C, Gil MJ, Cienfuegos JA, Salvador J, Frühbeck G** 2007 Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab 92:3719–3727
10. **Ginde SR, Geliebter A, Rubiano F, Silva AM, Wang J, Heshka S, Heymsfield SB** 2005 Air displacement plethysmography: validation in overweight and obese subjects. Obes Res 13:1232–1237
11. **Gubern C, López-Bermejo A, Biarnés J, Vendrell J, Ricart W, Fernández-Real JM** 2006 Natural antibiotics and insulin sensitivity: the role of bactericidal and permeability increasing protein (BPI). Diabetes 55:216–224
12. **Fernandez-Real JM, Lopez-Bermejo A, Vendrell J, Ferri MJ, Recasens M, Ricart W** 2006 Burden of infection and insulin resistance in healthy middle-aged men. Diabetes Care 29:1058–1064
13. **Gómez-Ambrosi J, Salvador J, Silva C, Pastor C, Rotellar F, Gil MJ, Cienfuegos JA, Frühbeck G** 2006 Increased cardiovascular risk markers in obesity are associated with body adiposity: role of leptin. Thromb Haemost 95:991–996
14. **Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ** 2000 Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85:2402–2410
15. **Gómez-Ambrosi J, Salvador J, Páramo JA, Orbe J, de Irala J, Diez-Caballero A, Gil MJ, Cienfuegos JA** 2002 Involvement of leptin in the association between percentage of body fat and cardiovascular risk factors. Clin Biochem 35:315–320
16. **Hinton PS, Rector RS, Thomas TR** 2006 Weight-bearing, aerobic exercise increases markers of bone formation during short-term weight loss in overweight and obese men and women. Metabolism 55:1616–1618
17. **Gill JM, Malkova D** 2006 Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. Clin Sci 110:409–425
18. **Holloszy JO** 2005 Exercise-induced increase in muscle insulin sensitivity. J Appl Physiol 99:338–343
19. **Cartee GD, Holloszy JO** 1990 Exercise increases susceptibility of muscle glucose transport to activation by various stimuli. Am J Physiol 258:E390—E393

20. **Gao J, Gulve EA, Holloszy JO** 1994 Contraction-induced increase in muscle insulin sensitivity: requirement for a serum factor. Am J Physiol 266:E186–E192
21. **Fisher JS, Gao J, Han DH, Holloszy JO, Nolte LA** 2002 Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. Am J Physiol Endocrinol Metab 282:E18–E23
22. **Viapiana O, Gatti D, Dalle Grave R, Todesco T, Rossini M, Braga V, Idolazzi L, Fracassi E, Adami S** 2007 Marked increases in bone mineral density and biochemical markers of bone turnover in patients with anorexia nervosa gaining weight. Bone 40:1073–1077
23. **Bowen J, Noakes M, Clifton PM** 2004 A high dairy protein, high-calcium diet minimizes bone turnover in overweight adults during weight loss. J Nutr 134:568–573
24. **Villareal DT, Fontana L, Weiss EP, Racette SB, Steger-May K, Schrechtman KB, Klein S, Holloszy JO** 2006 Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss. Arch Intern Med 166:2502–2510
25. **Ricci TA, Chowdhury HA, Heymsfield SB, Stahl T, Pierson Jr RN, Shapses SA** 1998 Calcium supplementation suppresses bone turnover during weight reduction in postmenopausal women. J Bone Miner Res 13:1045–1050