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**The relationship of serum osteocalcin concentration to
insulin secretion, sensitivity and disposal with hypocaloric diet and
resistance training**

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Abstract

Context: Bone has recently been described to exhibit 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 3 different studies in non-diabetic subjects: 1 cross-sectional in 149 men (using minimal model); and 2 longitudinal in 2 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 post-intervention 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, BMI 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.

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. 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 expenditure. 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

One hundred and forty nine consecutive men (mean age 50.2 ± 11.7 years, range 30-68 years; mean BMI 27.6 ± 3.5 kg/m²) 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/m², 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 < 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 intravenous glucose tolerance test (FSIVGTT) with minimal model analysis. *Insulin secretion* was calculated as the insulin area during the first 10 minutes of the FSIVGTT. 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 8:00 and 8:30 AM 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 minutes, after which glucose (300 mg/ kg body weight) was injected over 1 minute starting at time 0, and insulin (Actrapid, Novo, Denmark; 0.03 U/kg) was administered at time 20 minutes. Additional samples were

obtained from a contra-lateral antecubital vein up to 180 minutes.

Effects of slight weight loss with or without regular physical activity

Sedentary, non-smoking, obese (body mass index (BMI): 30-40 kg/m²) women, aged 40-60 years, 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 form before participating in the study. This project

was approved by the ethical committee of the regional health department.

Participants were randomized to 3 groups: a control group (C; n=7); a diet group (D; n=8) with a caloric restriction of 500 kcal/day; and a diet and resistance training group (D+RT; n=11) with the same caloric restriction as group D and a 16-week supervised resistance training program of 2 sessions/ week. During the 16 weeks 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.

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; Madison, WI). This diet was designed to elicit a 0.5 kg weight loss per week. The control group

was asked to maintain body weight. Throughout the 16-week intervention period, body weight was recorded every two weeks in both D and D+RT groups. Each subject of the intervention groups participated in a series of 1-hour seminars (every two weeks) 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%.

Resistance training 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 weeks to perform dynamic resistance exercise, for 45–60 min per session. A minimum of 2 days 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 weeks 0 and 16 by using 1-RM actions.

Magnetic Resonance (MR)

The volumes of visceral and abdominal subcutaneous adipose tissue were measured by magnetic resonance. MR imaging was performed with a 1T magnet (Magnetom Impact Expert, SIEMENS) 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. An FOV 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 minutes. MR imaging of the both thighs was then obtained. T1-weighted sequence was used with a repetition time (TR) of 645 ms and a spin echo time (TE) of 20 ms.

The field of view was 500 x 500 mm, and the matrix was 512 x 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 labelled using sagittal scout images, referred to discal level. We used a specially designed image analysis software (SliceOmatic 4.3 , Tomovision Inc, Montreal) for quantitative analysis of the images.

Effects of moderate weight loss

In order to further evaluate the effect of moderate weight loss on circulating osteocalcin after weight loss, 20 Caucasian obese volunteers (9 male, 11 female, age range 21 to 66 years) 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, co-morbidity evaluation as well as nutritional interviews performed by a multidisciplinary consultation team. All subjects were non-smokers. 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, California) 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, and 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, California, USA). 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 3 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 in order 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 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 standard deviations above the average OD at zero binding, was 0.4 ng/ml and the intra- and inter-assay CV were less than 10%. Serum adiponectin levels were measured by a commercially available enzyme-linked immunoassay (ELISA) kit (LINCO Research, MO, USA). The intra- and inter-assay 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 crossreactivity 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 analyser 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 coefficients of variation were similar to those previously reported (11,12).

In the *effects of slight weight loss with or without regular physical activity*, resting blood samples were drawn at weeks 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 coefficients of variation were less than 5%. To estimate insulin resistance, the HOMA index was calculated as fasting insulin concentration ($\mu\text{U/mL}$) \times fasting glucose concentration (mmol/L)/22.5.

In the *effects of moderate weight loss* study, plasma glucose was analyzed by an automated analyzer (Roche/Hitachi Modular P800) as previously described (13). Insulin was measured by means of an enzyme-amplified chemiluminescence assay (IMMULITE®, Diagnostic Products Corp., Los Angeles, CA, USA). An indirect measure of insulin sensitivity was calculated from the fasting plasma glucose and insulin concentrations by using the quantitative insulin sensitivity check index (QUICKI) (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 body mass index and waist-to-hip ratio. The models were built in a customized way by means of the enter method, that 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 co-linearity were solved by centring some of the variables. The statistical package used was Stata v.8. Levels of statistical significance were set at $P < 0.05$.

Results

Cross-sectional study

We evaluated 149 men, aged 50.2 ± 11.7 years, with mean BMI 27.6 ± 3.5 kg/m², a median insulin sensitivity of $2.35 \times 10^{-4} \text{ min}^{-1} \text{ mU/L}$ (interquartile range, 1.23-3.2), and a median insulin secretion of $359.1 \text{ mU/L} \cdot \text{min}^{-1}$ (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 ($r=0.23$, $p=0.006$, Figure 1). The statistical power of this association was 81% ($\alpha=0.05$, $\beta=0.80$).

Interestingly, the association appeared stronger in lean subjects (BMI < 25

kg/m²) (Figure 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, in which no action was taken (C; n=7). Baseline characteristics were similar in the 3 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 weeks, 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 (Figure 2). The statistical power of this change in serum osteocalcin was 96% ($\alpha=0.05$, $\beta=0.80$). This was observed in parallel with reduced visceral fat mass, unchanged thigh muscle mass, and increased leg strength and force (Figure 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 diet and diet plus RT, $n=19$) the post-intervention serum levels of osteocalcin were associated with both insulin resistance ($r=-0.49$, $p=0.03$) and fasting

triglycerides (Figure 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 (Figure 4). The statistical power of this change in serum osteocalcin was 78% ($\alpha=0.05$, $\beta=0.80$). 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 diet and diet + RT groups, $r=-0.50$, $p=0.02$, $n=19$) but not in the moderate weight loss group.

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 diet + exercise 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 diet + 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 were, to some extent, similar in the diet group and diet + RT group (the change in insulin resistance in the diet group did not reach statistical significance). However, the most striking differences between these groups were the change in leg force, that was strongly related with the change in serum osteocalcin in univariate and multivariate analysis. Thigh muscle mass was unchanged after diet + regular exercise in association with increased leg strength and force. In contrast, in the diet group, thigh muscle mass was significantly decreased after 16 weeks (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 a 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 post-exercise 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-beta-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 (Figure 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. reported no significant changes in osteocalcin levels with weight loss due to caloric restriction (24). 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 nerviosa, 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. reported that undercarboxylated osteocalcin was the active form of osteocalcin in rodent models (8).

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

No potential conflict of interest relevant to this article is reported.

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Figure Legends

Figure 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 as defined as having a BMI < 25 kg/m²; overweight as BMI ≥ 25 and < 30 kg/m²; obese as BMI > 30 kg/m². We used the non-transformed value of osteocalcin because the Kurtosis and skewness values were closer to 0 than the log-transformed value in this population.

Figure 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).

— Median
□ 25%-75%
┌ Min-Max

Figure 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 are only for round empty symbols in all panels.

Figure 4. Obese men and women (n=20) enrolled in the moderate weight loss intervention. Changes in BMI (left panel) and osteocalcin (right panel).

— Median
□ 25%-75%
┌ Min-Max

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

	Control			Diet			Diet + exercise			P*
n	7			8			11			
Age	51.5 ± 7.2			51.6 ± 6.6			47.7 ± 6.5			0.36
	Pre	Post	p	Post	p	Pre	Post	p	-	
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	46667.6 ± 7736	46465.5 ± 7167.4	0.74	48966.4 ± 10883.2	47226.8 ± 11609	0.039	46867.6 ± 9021.3	46470.2 ± 8839.2	0.87	0.87
Thigh fat area	88332 ± 19339	89127.2 ± 19090.9	0.59	87641.9 ± 21716.5	72697.6 ± 18365.1	0.004	104923.7 ± 16539.6	87479.1 ± 14200.8	<0.0001	0.11
Visceral AT	3370.78 ± 1228.42	3329.26 ± 1187.23	0.65	3243.75 ± 1085.33	2557.51 ± 1171.16	0.007	3211.30 ± 1232.09	2528.43 ± 1039.89	0.0001	0.96
Total AT	16833.4 ± 4183.2	16623.5 ± 3785.1	0.53	16973.7 ± 4787.1	13333.2 ± 4840.8	0.001	18205 ± 3337.9	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, 1 repetition maximum; *ANOVA p for baseline characteristics among groups.

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

<i>Change in osteocalcin</i>				
	Beta	p value	Beta	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
<i>Change in visceral fat</i>	-0.52	0.002	-0.46	0.005
<i>Change in leg muscle strength</i>			0.51	0.002
<i>Adjusted R²</i>		0.24		0.30

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

	Before weight loss	After weight loss	<i>P</i>
N	20	20	—
Age, y	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/l	5.6 ± 0.2	5.1 ± 0.1	0.043
Insulin, μU/ml	21.4 ± 5.2	11.8 ± 1.7	0.112
QUICKI	0.312 ± 0.009	0.341 ± 0.012	0.031
<i>Leptin</i> , ng/ml	39.2 ± 12.1	25.4 ± 10.2	0.044

To convert glucose to mg/dl, divide by 0.05551. BMI, body mass index; WHR, waist-to-hip ratio; QUICKI, quantitative insulin sensitivity check index.

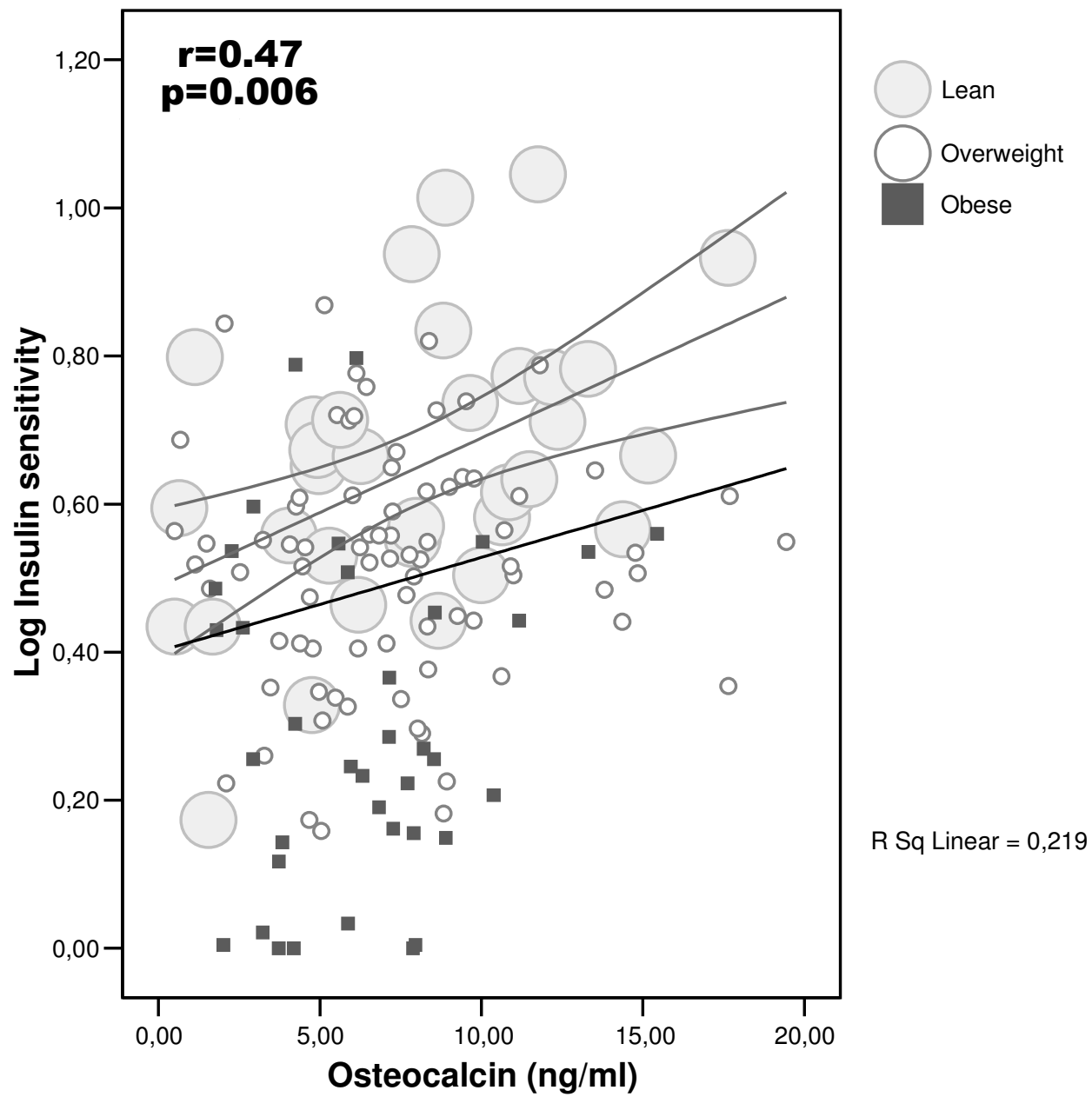
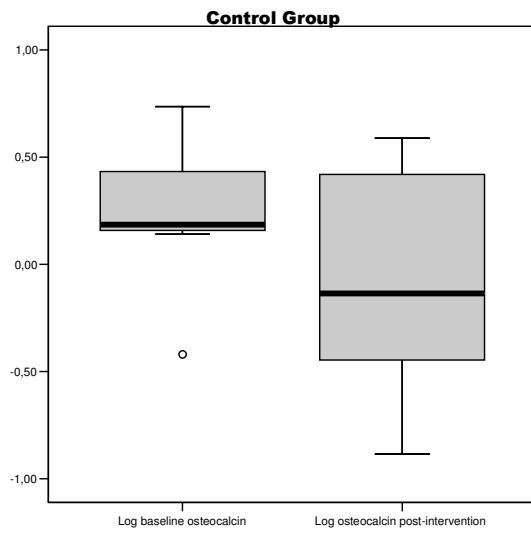
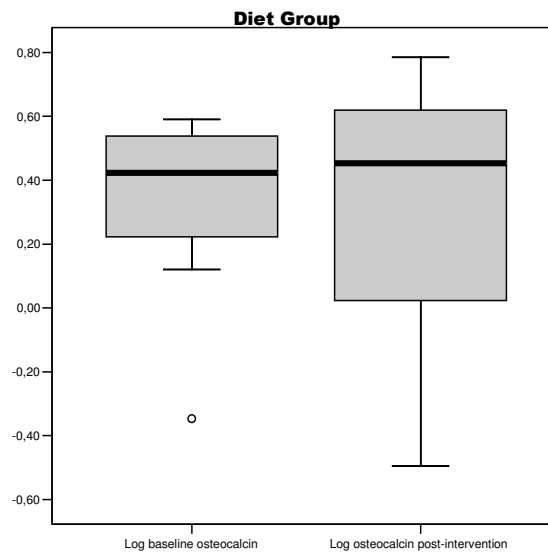


Figure 1

A



B



C

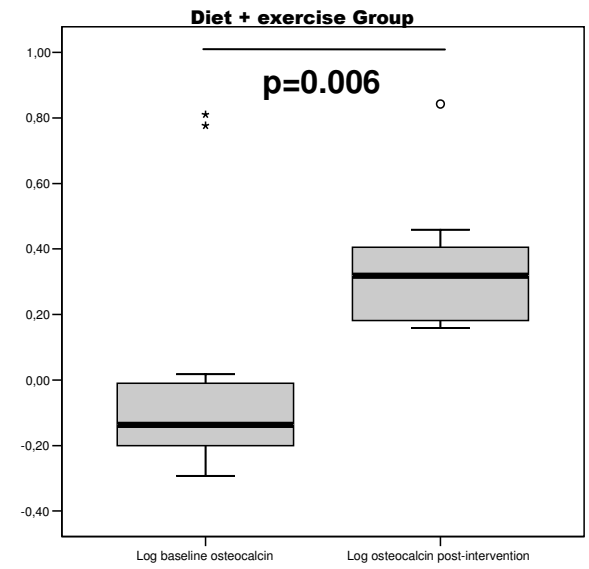


Figure 2

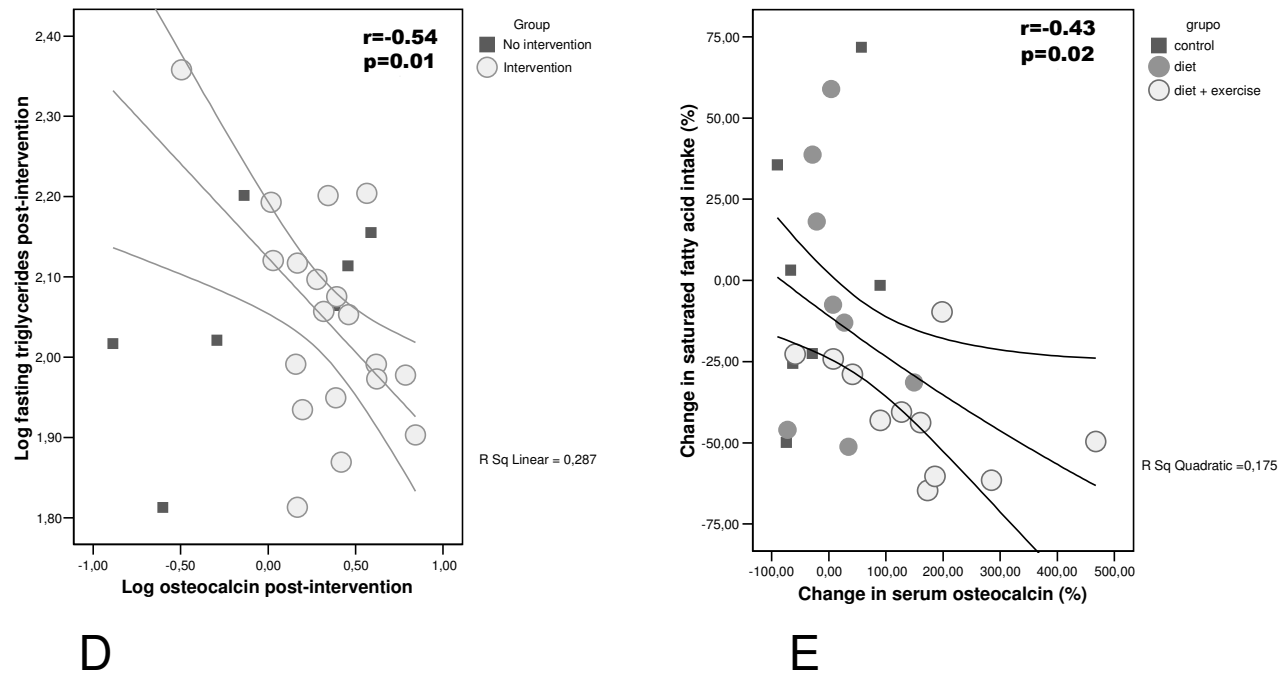
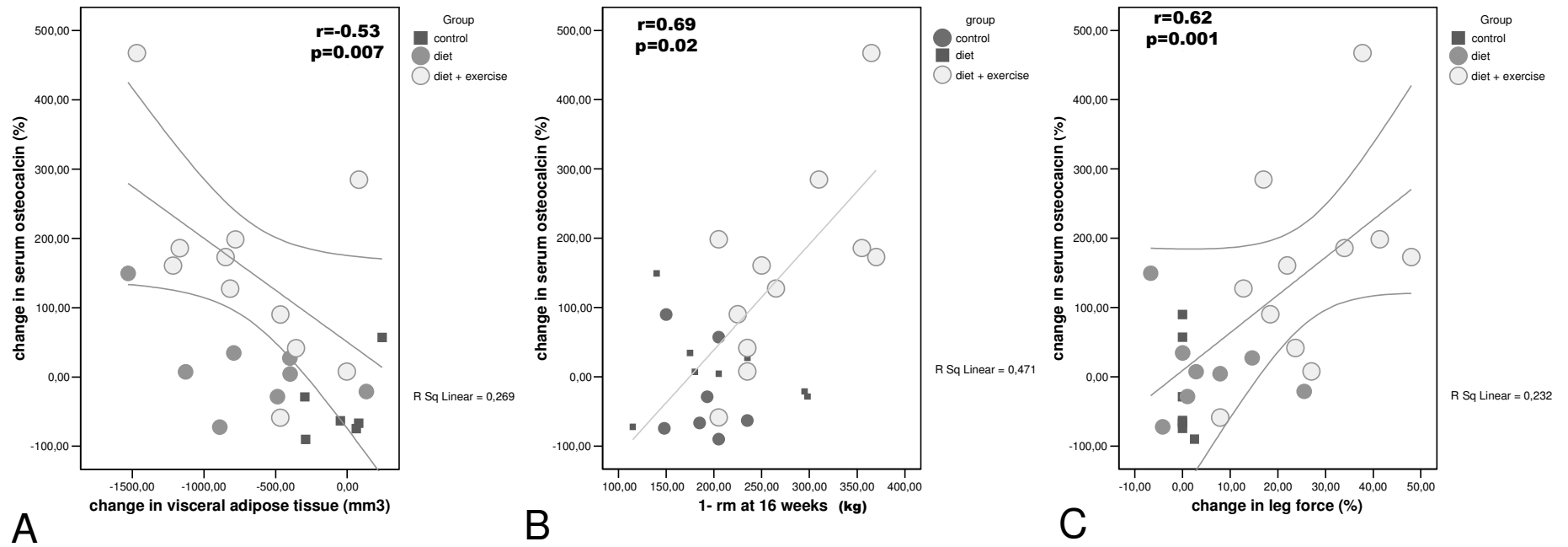


Figure 3

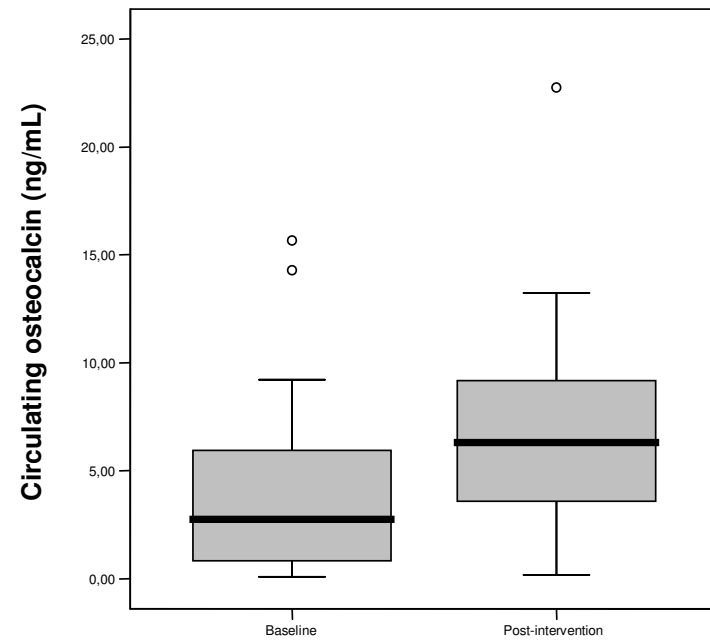
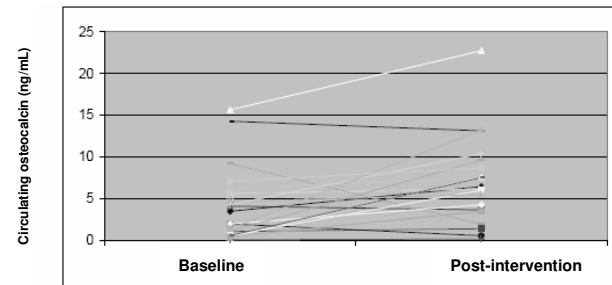
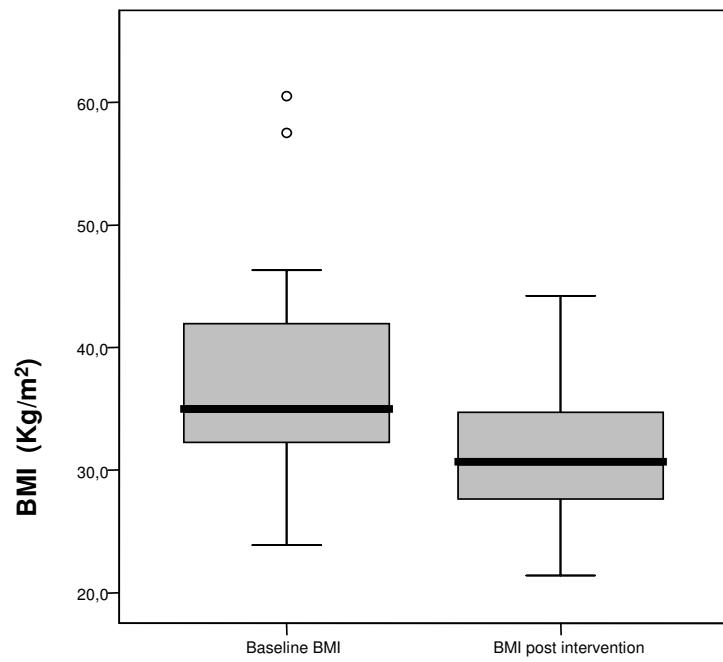


Figure 4