Changes in bone mineral density and bone turnover markers in obese women after short-term weight loss therapy during a 5-year follow-up

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KEY WORDS
- bone mineral density
- bone turnover
- obesity

INTRODUCTION
The protective effect of adiposity on bone metabolism has not been confirmed during long-term follow-up. It is not known whether the rate of bone turnover and changes in mineral metabolism in obese people result from endocrine properties of the adipose tissue or merely the mechanical load.

OBJECTIVES
The aim of the study was to evaluate bone and calcium-phosphorus metabolism in obese women during a 5-year follow-up.

PATIENTS AND METHODS
The study involved 47 obese women who underwent a 3-month weight loss therapy. We evaluated changes in the serum levels of parathormone (PTH), calcidiol (25(OH)D₃), collagen type I crosslinked C-telopeptide (CTx-I), osteocalcin, total calcium, inorganic phosphates, and in bone mineral density. The control group consisted of 17 healthy women with proper body weight.

RESULTS
We observed a similar decrease in bone mineral density (BMD) in the lumbar spine and femoral neck, and a comparable decrease in the serum levels of CTx-I and osteocalcin in both groups during the 5-year follow-up. Changes in serum PTH levels were not statistically significant. In obese women, a nonsignificant increase in the serum level of 25(OH)D₃ was observed as early as after a 3-month weight loss therapy and during follow-up. In controls, serum 25(OH)D₃ levels tended to decrease. During follow-up, the number of obese patients with disturbances in vitamin D metabolism decreased from 78.7% to 53.2% (P = 0.01). Such disturbances were observed in 35.3% of the control group. In obese patients, there was a positive correlation between the change in body mass and BMD in the proximal femur (r = 0.279, P = 0.04). In controls, there was a positive correlation between the change in body mass and BMD in the lumbar spine (r = 0.477, P = 0.05).

CONCLUSIONS
In obese women who underwent weight loss therapy, the levels of bone turnover markers decreased and abnormal vitamin D metabolism was still observed during the 5-year follow-up.

INTRODUCTION
It is widely recognized that obesity has a protective effect on the bone tissue.¹ An increase in the body mass index (BMI) is followed by an increase in bone mineral content and bone mineral density (BMD). Obese women have a lower risk of low-energy fractures compared with normal-weight women.² ³ Ravn et al.,⁴ when comparing the populations of obese and lean women of similar age, showed that increased body weight significantly reduces the risk of osteoporosis. In elderly people, obesity exerts a protective effect against fractures of the proximal
femur and distal forearm. This may be partly due to shock absorption properties of the adipose tissue, especially in relation to injuries that affect the proximal femur.

Higher BMD and a lower risk of osteoporosis in obese women is also partially explained by an increased production of estrogen and lower serum levels of sex hormone binding globulin, and thus higher levels of free hormones in this patient group. A number of studies have shown no protective effect of obesity on the development of osteoporosis. Goulding et al. observed an increased risk of fractures in children with higher fat content, and Zhao et al. proved that despite an increase in the adipose tissue, menopause is associated with an increased rate of bone loss. Furthermore, obese people are predisposed to disorders of calcium and phosphate homeostasis, which results from low physical activity, improper diet, and low UV exposure. Thus, the protective effect of obesity on bone tissues still raises concerns.

Recently, in order to assess fracture risk independently of BMD, the markers of bone turnover have been used. Their serum levels reflect bone turnover and rebuilding that take place in the skeletal system. The exact relationship between body weight and the rate of bone turnover has not been established so far. Both increased and stable serum levels of bone resorption markers and decreased serum levels of bone formation markers have been observed. In addition, the long-term results of weight-loss therapy and its effect on bone metabolism causes even greater controversy. Long-term follow-up studies of bone metabolism in obese patients have been rare. It is not known whether the rates of bone turnover and bone loss in obese patients are comparable to those observed in patients with normal weight. Lower levels of vitamin D and subsequent secondary hyperparathyroidism are observed more often in obese patients. It is not known whether these abnormalities are persistent, and whether calcium and vitamin D supplementation is needed in this patient group.

The aim of this study was to assess bone and calcium-phosphorus metabolism in obese women compared with controls during a 5-year follow-up.

PATIENTS AND METHODS The study involved 47 obese women. The effect of weight reduction on bone metabolism was assessed in patients treated in the "WAGA" Weight Management Clinic, Katowice, Poland, in the years 2003–2004. All patients were diagnosed with simple obesity without concomitant diseases. The inclusion criteria were as follows: stable body weight in the previous 3 months, normal lipid profile and blood glucose level, blood pressure ≤140/90 mmHg, no history of chronic inflammatory diseases or drug therapy (including drugs that affect bone metabolism). All patients were nonsmokers. All patients gave written informed consent. The study and the follow-up were approved by the Bioethics Committee of the Medical University of Silesia, Katowice, Poland.

All subjects participated in a 3-month weight-loss therapy that involved a 1000–1200 kcal/day balanced diet (daily calcium consumption of 500 mg), change of lifestyle, and regular physical exercise (60 min, 3 times a week). Each patient was examined every 2 weeks by a physician and a dietician. Each patient was asked to keep a food diary.

Clinical characteristics were recorded at baseline and after the therapy. Body weight and height were measured, and BMI was calculated. Body composition was analyzed using the bioimpedance method. After 5 years, patients were reinvited for anthropological measurement, densitometry, and biochemical and hormone tests. During the visit, data about comorbidities (hypertension, diabetes, dyslipidemia, thyroid disease, ischemic heart disease) and about the present therapy were collected.

In obese subjects, blood samples and anthropometric measurements were taken 3 times: at baseline, after the 3-month weight-loss therapy, and 5 years after the therapy. Blood samples (20 ml) were obtained from each subject in the morning between 8 and 9 a.m., after an overnight fast. After clot formation, the samples were centrifuged (1000 × g) at room temperature for 10 minutes. Serum was drawn into plastic vials and stored at −70°C until the assay. The serum levels of parathormone (PTH), 25(OH)D3, collagen type I cross-linked C-telopeptide (CTX-I), osteocalcin, total calcium, and phosphorus were determined.

BMD of the proximal femur and lumbar spine was measured twice using dual energy X-ray absorptiometry (DXA). Patients remained under the care of the "WAGA" Weight Management Clinic.

The control group consisted of 17 healthy women of similar age. Laboratory and anthropometric measurements were taken twice: at baseline and after 5 years.

Individual absolute fracture risk was estimated according to the World Health Organization (WHO) recommendations, using the FRAX™-WHO Fracture Risk Assessment Tool (http://www.shef.ac.uk/FRAX/tool.jsp). The characteristics of both groups are shown in TABLE 1.

Clinical and metabolic characteristics Weight and height were measured using electronic medical scale (RADWAG, Poland). BMI was calculated using the formula: BMI = body weight (kg)/height (m²). Waist circumference was measured half way between the lower costal margin and superior iliac crest. Body weight composition was analyzed using the bioimpedance method (Bodystat 1500, Bodystat Ltd., Great Britain). Blood pressure was measured using a standard mercury sphygmomanometer cuff (12 × 23 cm), on the left forearm, after 5 minutes in a sitting position.
independent variables and the Wilcoxon pair-wise comparison for dependent variables were used as appropriate. Changes in the parameters over time were evaluated by the analysis of variance. The correlation coefficients (r) was calculated according to the Spearman’s rank correlation coefficient. P < 0.05 was considered statistically significant.

Results

Women with simple obesity had higher BMD in the examined areas, a lower 10-year absolute fracture risk (FRAX™), lower serum levels of osteocalcin, 25(OH)D₃, and phosphate, but higher serum levels of PTH compared with controls (Table 1).

After the 3-month weight loss therapy, we observed a decrease in body mass (by 9.5 ±4.2%) and body fat mass (by 12.6 ±8.3%). In addition, we noted a decrease in the serum levels of total cholesterol (9.9 ±9.0%), low-density lipoprotein cholesterol (10.3 ±17.3%), and triglycerides (10.6 ±30.5%). Five years after the therapy, body mass was only 0.5 ±7.8% lower compared with the baseline values, and fat content increased by 0.6 ±6.9%.

After 5 years, we still observed higher BMD, a lower 10-year absolute fracture risk (FRAX™),

Laboratory tests

The laboratory tests were performed at the Isotope Laboratory of the Department of Nephrology, Endocrinology and Metabolic Diseases and Department of Pathophysiology, Medical University of Silesia, Katowice, Poland. An electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics GmbH, Germany) was used for PTH, osteocalcin, and CTx-I assays. 25(OH)D₃ was measured using a radioimmunoassay (Bio Source ‑EUROPE S.A., Nivelles, Belgium). The levels of total calcium, inorganic phosphate, serum glucose, and lipids were measured using a spectrophotometer (Point Scientific Inc., Michigan, United States).

Statistical analysis

The data are presented as mean ± standard deviation. The analyses were performed using the STATISTICA 8.0 software (StatSoft Polska, Krakow, Poland). Normality of distribution was tested with the Kolmogorov ‑Smirnov test. Due to the size of the control group and the distribution of some of the tested parameters, the U Mann-Whitney pair-wise comparison for dependent variables were used as appropriate. Changes in the parameters over time were evaluated by the analysis of variance. The correlation coefficients (r) was calculated according to the Spearman’s rank correlation coefficient. P < 0.05 was considered statistically significant.

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After 5 years, we still observed higher BMD, a lower 10-year absolute fracture risk (FRAX™),
and higher serum PTH levels in obese patients compared with controls (Table 1).

In both groups, a similar reduction in BMD of the proximal femur and lumbar spine was observed during the 5-year follow-up (Table 1).

In both groups, a decrease in the serum levels of CTx-I and osteocalcin was observed. Changes in the serum levels of PTH and 25(OH)D$_3$ did not reach statistical significance (Table 1). In obese women, there was a slight increase in serum 25(OH)D$_3$ levels, which occurred after the weight loss therapy and was still observed during follow-up (Table 1). In controls, 25(OH)D$_3$ decreased. These changes, observed during the 5-year follow-up were statistically significant (Table 2).

Serum 25(OH)D$_3$ levels of 0 to 10 ng/ml were considered as “deficit”, of 10 to 20 ng/ml – deficiency, and of 20 to 30 ng/ml – hypovitaminosis. At baseline, deficit was recognized in 3 obese patients (6.4%), deficiency in 15 (13.9%), and hypovitaminosis in 19 (40.4%). After 5 years, no deficit of 25(OH)D$_3$ was found in the study group, but deficiency was observed in 15 obese patients (13.9%) and hypovitaminosis in 10 (21.3%). The percentage of subjects with abnormal vitamin D metabolism decreased from 78.7% to 53.2% (P = 0.01). In the control group, such abnormalities were less common. Deficiency was recognized in 2 subjects (11.8%) and hypovitaminosis in 4 (23.5%).

Correlations In obese women, there was a positive correlation between the change in body weight and the change in BMD of the proximal femur (r = 0.279, P = 0.04). In contrast, in the control group, there was a positive correlation between the change in body weight and the change in BMD of the lumbar spine (r = 0.477, P = 0.05).

Additionally, in obese women, correlations of borderline significance between serum 25(OH)D$_3$ levels and body weight (r = −0.28, P = 0.06) and waist circumference (r = −0.27, P = 0.07) were observed, but these relationships were no longer present after 5 years.

**TABLE 2** Comparison of changes in the assessed parameters in the study and control groups during 5-year follow-up

<table>
<thead>
<tr>
<th></th>
<th>Study group n = 47</th>
<th>Control group n = 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ body mass, %</td>
<td>−0.5 ± 7.9</td>
<td>−1.3 ± 3.9</td>
</tr>
<tr>
<td>Δ BMD L2–L4, g/cm$^2$</td>
<td>−0.03 ± 0.11</td>
<td>−0.07 ± 0.14</td>
</tr>
<tr>
<td>Δ BMD proximal femur, g/cm$^2$</td>
<td>−0.05 ± 0.09</td>
<td>−0.02 ± 0.08</td>
</tr>
<tr>
<td>Δ fat tissue, %</td>
<td>0.6 ± 6.9</td>
<td>3.2 ± 7.9</td>
</tr>
<tr>
<td>Δ lean tissue, %</td>
<td>−0.6 ± 7.7</td>
<td>−3.6 ± 10.9</td>
</tr>
<tr>
<td>Δ osteocalcin, ng/ml</td>
<td>−1.5 ± 6.5</td>
<td>−1.1 ± 13.5</td>
</tr>
<tr>
<td>Δ PTH, pg/ml</td>
<td>−6.2 ± 19.7</td>
<td>0.1 ± 20.6</td>
</tr>
<tr>
<td>Δ CTx-I, ng/ml</td>
<td>−0.04 ± 0.1</td>
<td>−0.05 ± 0.14</td>
</tr>
<tr>
<td>Δ 25(OH)D$_3$, ng/ml</td>
<td>3.7 ± 11.3$^a$</td>
<td>−6.4 ± 17.4$^a$</td>
</tr>
</tbody>
</table>

statistical significance vs. controls: $^a$ P = 0.01

Abbreviations: see Table 1

**DISCUSSION** The present study is one of the few long-term follow-up studies evaluating the changes in BMD and bone turnover markers in obese patients. Fogelholm et al. evaluated changes in BMD in obese women (before menopause) after a 3-month weight-loss program and regain of body weight during a 36-month follow-up. The authors described a slight decrease in BMD at 3 months that was partly neutralized after weight regain. Also, Compston et al. observed in a small group of obese women undergoing a 10-week weight-loss therapy that BMD returned to baseline after 10 months, during which the women regained their weight. However, in both studies, neither bone turnover markers nor the serum levels of calcitropic hormones were assessed.

In our study, we observed significant differences in the serum levels of calcitropic hormones, osteocalcin, and BMD between obese patients and controls. Higher serum PTH levels in obese women can be explained by lower levels of vitamin D, which reduces calcium absorption from the gastrointestinal tract, and by increased calcium renal excretion. These processes reduce the serum levels of ionized calcium that stimulates the parathyroid glands.

According to McCarthy et al., PTH may promote weight gain by inhibiting lipolysis stimulated by catecholamines in adipocytes. Thus, obesity may be an essential factor associated with increased secretion of PTH, and PTH enhances the growth of the adipose tissue. The results of Andersen et al., who described the association between PTH levels and the degree of obesity many years ago, may support this thesis. Serum PTH levels in our study group were increased and remained so during the 5-year follow-up, as compared with the control group.

Lower serum 25(OH)D$_3$ levels in obese women may be explained by excessive storage of 25(OH)D$_3$ in the adipose tissue, inhibition of 25(OH)D$_3$ synthesis in hepatocytes by 1,25(OH)$_2$D$_3$ (its levels are elevated in obesity), and lower physical activity, which is also associated with lower sun exposure. An increase in serum 25(OH)D$_3$ levels after weight loss and a stronger correlation of these levels with fat content than with the BMI may confirm these hypotheses. In our study, serum 25(OH)D$_3$ levels increased after 5 years, but the increase was not statistically significant, probably due to a small size of the study group.

We analyzed serum 25(OH)D$_3$ levels in terms of the recommended concentrations and found significant abnormalities (deficit, deficiency, and hypovitaminosis) in 78.7% of the patients. For comparison, deficiency or hypovitaminosis was found in 35.3% of the control group. After 5 years, the percentage of obese patients with abnormal vitamin D metabolism decreased to 53.2%. Deficit was no longer observed, only deficiency was found in 15 patients (13.9%) and hypovitaminosis in 10 (21.3%). The observed changes might have resulted from a change in diet (higher intake of vitamin D and dairy products) and in lifestyle (increased physical activity) after weight...
loss therapy and a consultation with a dietician. However, lifestyle modification and sun exposure cause a smaller increase in serum 25(OH)D$_3$ levels than oral vitamin D supplementation. After oral administration of vitamin D, an increase in serum 25(OH)D$_3$ levels in obese patients is lower than in lean ones, which could indicate impaired absorption of standard doses of this vitamin.

Adequate vitamin D supplementation is important because reduced serum 25(OH)D$_3$ levels (<12 ng/ml) decrease serum 1,25(OH)$_2$D$_3$, PTH, and osteocalcin. It is known that in winter, reduced serum 25(OH)D$_3$ levels are associated with increased serum levels of PTH and osteocalcin. However, fluctuation in 25(OH)D$_3$ levels does not result in normalization of PTH levels during summer.

In obese women, baseline serum levels of osteocalcin were lower compared with controls. During the 5-year follow-up, the serum levels of CTx-I and osteocalcin decreased, indicating slow bone turnover in obese patients. Similar findings were reported by Cifuentes et al. who observed lower serum osteocalcin levels in obese postmenopausal women. However, there have been some discrepancies. Ostrowska et al. described increased serum levels of bone turnover markers, both formation and resorption, in women with the BMI above 40 mg/m$^2$. Hyldestrup et al. observed stable levels of bone resorption markers and decreased levels of bone formation markers. In controls, the serum levels of bone turnover markers remained stable. The effects of body weight on the bone tissue and its metabolism are also not clear. It has been shown that weight gain is associated both with increased bone mass and with reduced bone loss that occurs with age.

Unlike people with normal weight, obese patients have increased BMD both in the areas of mechanical loading (femoral neck) and in the areas where such loading is not present (distal part of the radius). However, there is no agreement as to whether fat or lean body tissue determines the development of bone mass. Both positive (reduced risk of osteoporosis) and negative (no protection against bone mass loss) effects of fat tissue on the bone have been reported.

In our study, we observed both increased BMD and a lower 10-year absolute fracture risk in obese women. These ratios were maintained during the 5-year follow-up, despite a decrease in BMD around the femoral neck and lumbar spine in both patient and control groups (by 5% and 3%, and by 7% and 2%, respectively). We also did not observe a relationship between fat content and BMD, which can be due to a limited number of subjects studied in the present investigation. The relationship between fat content and bone tissue mineralization is weaker than that between body mass and bone mineralization. In obese patients, we observed a relationship between body mass and BMD in the femoral neck, and in the control group there was a correlation between body mass and BMD in the lumbar spine. These results suggest that mechanical loading is a more important determinant of bone mass (bone formation stimulation) than the adipose tissue. Because adipose tissue constitutes less than 40% of the total body weight on average, mechanical loading associated with increased fat mass may be insufficient to explain the effect of fat mass on the bone tissue.

To summarize, our most important findings were the reduced levels of bone turnover markers and abnormal vitamin D metabolism observed in obese women during the 5-year follow-up. Therefore, it seems vital to provide obese patients with adequate vitamin D supplementation. It may be a way of slowing bone resorption and preventing secondary hyperparathyroidism. Due to metabolic factors, obese patients may require higher doses of vitamin D.

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REFERENCES


ARTYKUŁ ORYGINALNY

Zmiany gęstości mineralnej kości oraz wskaźników obrotu kostnego u otyłych kobiet poddanych krótkotrwałemu odchudzaniu w czasie pięcioletniej obserwacji

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STRZESZCZENIE

WPROWADZENIE Ochronnego wpływu tkanki tłuszczowej na metabolizm tkanki kostnej nie potwierdzono w obserwacji odległej. Nie wiadomo, czy zmiany metabolizmu oraz szybkość przemian mineralnej kości u osób otyłych są następstwem działania endokrynego tkanki tłuszczowej, czy jedynie obciążenia mechanicznego.

CELE Celem badania była ocena metabolizmu kości i gospodarki wapniowo-fosforanowej u otyłych kobiet w czasie 5-letniej obserwacji.

PACJENCI I METODY Przeprowadzono badania u 47 otyłych kobiet, poddanych 3-miesięcznej kuracji odchudzającej. Oceniano zmiany stężenia w surowicy: parathormonu (PTH), kalcidiolu (25(OH)D₃), C-końcowego telopeptydu kolagenu typu I (collagen type I crosslinked C‑telopeptide – CTx-I), osteokalcyny, wapnia całkowitego, fosforanów nieorganicznych oraz gęstości mineralnej kości (bone mineral density – BMD), wykorzystując metodę absorpcjometrii podwójnej energii promieniowania rentgenowskiego. Grupę kontrolną stanowiło 17 zdrowych kobiet z prawidłową masą ciała.

WYNIKI W obu badanych grupach w okresie 5 lat stwierdzono porównywalne zmniejszenie BMD w zakresie kręgosłupa lędźwiowego i szyjki kości udowej oraz również porównywalne zmniejszenie się stężeń CTx-I i osteokalcyny w surowicy. Zmiany stężenia PTH nie osiągnęły znamienności statystycznej. W grupie otyłych kobiet obserwowano nieznamienny wzrost stężenia 25(OH)D₃ w surowicy, który wystąpił już po 3 miesiącach odchudzania i utrzymał się w dalszej obserwacji. W grupie kontrolnej stężenie 25(OH)D₃ w surowicy wykazywało tendencję spadkową. Podczas obserwacji liczba otyłych pacjentek z zaburzeniami gospodarki witaminą D zmniejszyła się z 78,7% do 53,2% (P = 0,01). W grupie kontrolnej zaburzenia gospodarki witaminą D stwierdzono u 35,3% osób. W grupie badanej stwierdzono dodatnią korelację pomiędzy zmianą masy ciała i BMD w obrębie szyjki kości udowej (r = 0,279; P = 0,04). W grupie kontrolnej stwierdzono dodatnią korelację pomiędzy zmianą masy ciała i BMD w obrębie odcinka lędźwiowego kręgosłupa (r = 0,477; P = 0,05).

WNIOSKI U otyłych kobiet w okresie 5-letniej obserwacji po leczeniu zmniejszającym masę ciała stwierdzono zmniejszanie się stężeń wskaźników przemian mineralnej kości oraz utrzymywanie się zaburzeń gospodarki witaminą D.

SŁOWA KLUCZOWE gęstość mineralna kości, otyłość, przemiana mineralna kości

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