Interaction between bone, the neuroendocrine system and metabolism
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Effects of parathyroid hormone-induced increase in osteocalcin on glucose metabolism and plasma testosterone in men

Eelkje J. Limonard, Nathalie van der Velde, Mariette T. Ackermans, Annemieke C. Heijboer, Eric Fliers, Peter H. Bisschop

Manuscript in preparation
Abstract

Background Osteocalcin is a protein produced by osteoblasts and a well-known marker of bone formation. Animal studies have identified osteocalcin, in particular its undercarboxylated form, to have an important role in energy metabolism and reproduction. In humans, the majority of cross-sectional studies show a negative association of (undercarboxylated) osteocalcin levels with blood glucose, insulin resistance, diabetes, obesity and markers of metabolic syndrome, while only limited data are available on the role of osteocalcin in gonadal function.

Objective We investigated the effect of changes in osteocalcin levels, induced by 12 weeks of parathyroid hormone (PTH) (1-34) 20μg/day treatment, on glucose tolerance, insulin sensitivity and plasma testosterone levels in men with osteoporosis.

Methods Four male subjects aged 50-80 years with an indication for pharmacological treatment of osteoporosis were included in this crossover intervention trial. Subjects were randomized to receive treatment with PTH (1-34) during 12 weeks followed by no treatment during 12 weeks or vice versa. At the end of each treatment arm osteocalcin concentrations as well as body composition, glucose metabolism and insulin sensitivity were measured by whole-body dual-energy x-ray absorptiometry (DXA) scanning, an oral glucose tolerance test and a two-step hyperinsulinemic euglycemic clamp.

Results In all subjects, osteocalcin concentrations increased during PTH (1-34) treatment. The median increase in osteocalcin was 127% (range, 84 to 191%). Despite these marked changes in osteocalcin levels, there was no significant change in glucose tolerance, insulin sensitivity, body composition or testosterone levels.

Conclusion The preliminary results from this study do not support a role for osteocalcin in the regulation of glucose homeostasis and testosterone levels in humans. However, the sample size of this intervention study is too small to draw definitive conclusions at this stage.

Introduction

Osteocalcin is an osteoblast-specific secreted molecule and a well-known marker of bone formation. In rodents, osteocalcin, in particular its undercarboxylated form, has been identified to have important hormonal functions, with a role in energy metabolism and reproduction (1-5). Osteocalcin knockout mice present with a metabolic phenotype of glucose intolerance, insulin resistance, increased fat mass and decreased energy expenditure (3). In addition, male osteocalcin knockout mice breed poorly and are characterized by decreased testicular and epididymal weight, decreased sperm counts and low circulating testosterone levels (1, 2). The recognition of the endocrine function of osteocalcin in mice resulted in a growing interest in the metabolic role of osteocalcin in humans. In two recent systematic surveys of the liter-
ature, the majority of the cross-sectional studies found a negative correlation of serum undercarboxylated or total osteocalcin levels with blood glucose, insulin resistance, diabetes, obesity and markers of metabolic syndrome (6, 7). To date, only three studies have investigated the effects of interventions in bone metabolism affecting osteocalcin concentrations on glucose metabolism (8-10). And only a few studies investigating the endocrine role of osteocalcin in human gonadal function are currently available and these studies reported conflicting results (11-17). The first genetic evidence that osteocalcin is involved in human gonadal function came from a loss-of-function mutation in the osteocalcin receptor found in two subjects during a systematic genomic analysis of subfertile males (1).

Because of the correlative nature of the majority of the currently available literature in humans, the presence of different forms of osteocalcin, and the different metabolic end points used, it is difficult to draw any definitive conclusions at this point. There is an urgent need for prospective intervention studies investigating the endocrine role of (undercarboxylated) osteocalcin in humans. Unfortunately, osteocalcin is not available for administration in humans, but endogenous osteocalcin was reported to increase twofold during daily parathyroid hormone (PTH) administration (9). Although mRNA encoding the PTH receptor has been found in human pancreatic and testis tissue (18), there are no studies available stating that the PTH receptor is expressed by these tissues and that PTH administration has direct effects on insulin signaling and plasma testosterone levels.

To further elucidate the hormonal actions of osteocalcin, we performed a prospective intervention study, in which we studied the effect of changes in osteocalcin levels, induced by PTH (1-34) treatment, on testosterone levels, glucose tolerance and insulin sensitivity in male subjects with primary osteoporosis.

**Subjects and methods**

**Subjects**

Male subjects aged 50-80 years with an indication for medical treatment of osteoporosis were recruited for this study. Indication for treatment was defined as having a T-score between -3.5 and -2.5, a recent (clinical) vertebral fracture or two prevalent vertebral fractures independent of bone mineral density, or a recent fracture and T-score ≤ -2.0. Exclusion criteria for participation were 1] contraindication to parathyroid hormone therapy: hypersensitivity to the active substrate or to any of the excipients, pre-existing hypercalcaemia, hepatic- or renal insufficiency, metabolic bone diseases other than primary osteoporosis or glucocorticoid-induced osteoporosis, unexplained elevations of alkaline phosphatase, prior external beam or implant radiation therapy to the skeleton, patients with skeletal malignancies or bone metastases; 2] any medication or disease influencing bone turnover; 3] diabetes mellitus and 4] hypogonadism. Subjects were recruited by advertisements in public spaces and hospital and general physician waiting areas. All subjects agreed to participate in the study and written informed consent was obtained from all participants.
Study design and intervention
This open-label crossover randomized trial was performed at the Department of Endocrinology and Metabolism of the Academic Medical Center (AMC) of the University of Amsterdam in the Netherlands. At baseline, we took a complete (family) history, performed a general physical examination and measured body weight, height and blood pressure. A venous blood sample was drawn after an overnight fast to determine serum concentrations of calcium, albumin, phosphate, parathyroid hormone, 25-hydroxyvitamin D (25(OH)D), alkaline phosphatase, creatinine, glucose, glycated hemoglobin, insulin, luteinizing hormone (LH), testosterone and sex hormone-binding globulin (SHGB). We also determined serum C-terminal crosslinking telopeptides of collagen type I (CTx), procollagen type 1 propeptide (P1NP) and osteocalcin as markers of bone turnover. After inclusion subjects were randomized to receive (1) PTH (1-34) 20μg daily (Forsteo, Eli Lily, the Netherlands) during 12 weeks followed by no treatment during 12 weeks or (2) no treatment for 12 weeks followed by 12 weeks of PTH (1-34) 20μg daily using a computer-generated randomization list (nQuery Advisor version 7.0, Statistical Solutions, Cork, Ireland). PTH (1-34) was injected subcutaneously in the morning or evening (depending on the subjects’ preference) at a fixed time during the entire intervention period. All participants received daily supplements of calcium carbonate (elemental calcium 500 mg) and cholecalciferol (800 IU). Every six weeks serum or plasma concentrations of glucose, insulin, LH, testosterone, CTx, P1NP and osteocalcin were determined. Body composition was measured at 12 and 24 weeks by whole-body dual-energy x-ray absorptiometry (DXA) scanning (Hologic Discovery, Bedford, MA, USA; APEX system software version 3.3). Glucose metabolism and insulin sensitivity were measured on two separate days using an oral glucose tolerance test (OGTT) and a two-step hyperinsulinemic euglycemic clamp at 12 and 24 weeks. The study was approved by the Institutional Review Board of the Academic Medical Center (AMC) in Amsterdam, and was carried out in compliance with the Helsinki Declaration. The study was registered at the Netherlands Trial Registry (http://www.trialregister.nl) as NTR3859.

Oral glucose tolerance test
Glucose tolerance was determined by an oral glucose tolerance test. Following an overnight fast an intravenous catheter was inserted in the antecubital vein of the right arm for blood sampling. To keep the sampling line patent, a slow infusion of NaCl 0.9% was used. At baseline a blood sample was drawn to determine glucose, insulin and C-peptide concentrations. Next, subjects were instructed to drink 75 grams of glucose dissolved in 300 ml of water within 60 seconds (t=0). The following four hours blood samples were drawn at t=5, 10, 15, 20, 40, 60, 80, 100, 120, 150, 180, 210 and 240 minutes for measurement of glucose, insulin and C-peptide concentrations. Subjects were restricted to bed rest with minimal physical activity.

Two-step hyperinsulinemic euglycemic clamp
Insulin sensitivity was measured with a two-step hyperinsulinemic euglycemic clamp. After an overnight fast subjects were admitted to the Metabolic Clinical Research Unit of the AMC at 8:00 h. For the administration of the stable isotope, glucose and insulin and for blood sampling, an intravenous catheter was inserted into the dorsal vein of each arm. One catheter was used to sample arterialized venous blood using a thermo-regulated hand box (60 °C). To keep the
sampling line patent, a slow infusion of NaCl 0.9% was used. The other catheter was used to infuse [6,6-²H2]glucose, a 20%-glucose solution and insulin. At 8:30 h, after taking a blood sample to determine the background enrichment of plasma glucose, a primed continuous infusion of [6,6-²H2]glucose (> 99% enriched; Cambridge Isotopes, Andover, USA) was started at a rate of 0.11 μmol/kg·min (priming dose: 11 μmol/kg). After a two hour equilibration period blood samples were drawn for measurement of glucose enrichment and glucoregulatory hormones (insulin, glucagon and cortisol). Thereafter, a primed continuous infusion of insulin (Actrapid 100 U/ml; Novo Nordisk Farma BV, Alphen aan den Rijn, the Netherlands) was started at a rate of 15 mU/m2 body surface area·min to assess hepatic insulin sensitivity (step 1). Plasma glucose concentrations were measured every 10 minutes and the 20%-glucose solution was infused at a variable rate to maintain euglycemia at 5 mmol/L. [6,6-²H2]glucose was added to the infusate containing the 20%-glucose solution to achieve glucose enrichments of 1%. This was done to minimize changes in isotopic enrichment from changes in the infusion rate of exogenous glucose and thus to allow for accurate quantification of endogenous glucose production. After two hours blood samples were drawn at 5 min intervals for glucose enrichment and glucoregulatory hormones. Thereafter the infusion rate of insulin was increased to 60 mU/m2 body surface area·min to assess peripheral insulin sensitivity (step 2). After 2 h blood samples were drawn again at 5 min intervals for glucose enrichment and glucoregulatory hormones. Endogenous glucose production (EGP) and peripheral glucose uptake (rate of disappearance [Rd]) were calculated using modified versions of the Steele equations for the non-steady state and are expressed as micromoles/kilograms/minute (19, 20). Hepatic insulin sensitivity was expressed as percent insulin-mediated suppression of baseline EGP.

**Body composition**
Body composition was measured by whole-body dual-energy x-ray absorptiometry (DXA) scanning (Hologic Discovery, Bedford, MA, USA; APEX system software version 3.3).

**Biochemical assessment**
CTx, P1NP and osteocalcin were measured using immunoassays (Modular Analytics E 170, Roche Diagnostics Corporation, Indianapolis, IN, Orion Diagnostica, Espoo, Finland, and BioSource, Nivelles, Belgium, respectively). Plasma glucose was determined with a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barleben, Germany). Glycated hemoglobin was determined with liquid chromatography with an automated glycohemoglobin analyzer (Tosoh Corporation, Tokyo, Japan). Insulin (chemiluminescent immunometric assay) and cortisol (chemiluminescent immunoassay) were measured on an IMMULITE 2000 system (Siemens Healthcare, Erlangen, Germany). Glucagon and C-peptide levels were determined with a ¹²⁵I radioimmunoassay (Merck Millipore, Darmstadt, Germany). [6,6-²H2]glucose enrichment was measured with gas chromatography-mass spectrometry, as previously described in detail (21). Testosterone was determined with an in-house UPLC-tandem MS method on an Acquity-Xevo TQ-S system (Waters, Etten-Leur, the Netherlands). LH was determined with an electrochemiluminescence immunoassay (Roche Diagnostics Limited, Rotkreuz, Switzerland). Inter-assay coefficients of variation (CV) were as follows: CTx 3%, P1NP 8%, osteocalcin 8%, glucose
3.9%, glycated hemoglobin 0.4%, insulin 9.2%, cortisol 8.4%, glucagon 6.6%, C-peptide 12.6%, [6,6-H2]glucose 1.4%, testosterone 1.5% and LH 1.7%. All blood samples during follow-up were collected in the morning between 7:00 and 9:00 h after an overnight fast. Blood samples were centrifuged within 30 min and analyzed immediately or stored at -80 °C until analysis. All assays were performed in duplicate at the end of the study period in a single batch.

**Statistical analysis**

The statistical analysis was carried out with SPSS for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). Data are presented as mean and standard deviation (mean±SEM) or median and range. Area under the curve (AUC) and incremental AUC (iAUC) of plasma glucose and insulin levels following the 75 g oral glucose load were calculated with GraphPad Prism for Windows (version 5.01; GraphPad Software, Inc.; La Jolla, CA, USA). Comparison between osteocalcin concentrations after 12 weeks of PTH (1-34) and 12 weeks of no intervention were made with the Wilcoxon signed-rank test. Non-parametric analysis was chosen due to the relatively small number of study subjects. Statistical tests were two-sided and a p-value of 0.05 was considered significant.

**Power and sample size calculation**

A previous study showed that median osteocalcin levels increase 177% (95% CI 139-232%) with three months of PTH (9). Based on these results, with a two-sided significance level of 0.05, a sample size of six will have 80% power to detect a significant difference in osteocalcin levels (nQuery Advisor 7.0, Janet D. Elashoff). For our primary outcome, the influence of osteocalcin on testosterone levels and glucose metabolism, no pilot studies have been performed, so an exact sample size calculation cannot be performed. Because of uncertainties in our calculation an extra two subjects (total of eight study subjects) will be included in the study.

**Results**

**Subject characteristics**

The results presented in this paper are based on an interim analysis of the data of four of the intended eight subjects to be included in this study. Table 1 shows the characteristics of the study participants. One subject was a current moderate smoker. None of the subjects used more than seven units of alcohol per week. Median 25-hydroxyvitamin D concentration was 43 nmol/L (range, 34 to 48 nmol/L). Two subjects had impaired fasting plasma glucose levels, i.e. > 5.6 mmol/L, but normal glycated hemoglobin concentrations. Median fasting plasma insulin levels were 45.5 pmol/L (range, 20.8-86.0 pmol/L). No adverse events occurred during the intervention period.
Bone turnover markers
Bone turnover marker concentrations increased with daily PTH (1-34) treatment (Table 2). The median increase in osteocalcin was 127% (range, 84 to 191%) (p = 0.068). An increase in CTx was observed after 12 weeks of PTH (1-34) in all except one subject (median increase 95 ng/L [range, -32 to 275 ng/L]). P1NP concentrations increased in all subjects (median increase 23 μg/L [range, 11 to 60 μg/L]).

Table 1 Characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 (51-73)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.3 (19.4-29.8)</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>1</td>
</tr>
<tr>
<td>Fracture (n)</td>
<td>4</td>
</tr>
<tr>
<td>DXA T-score femoral neck</td>
<td>-2.1 (-1.9 - -2.4)</td>
</tr>
<tr>
<td>DXA T-score L1-L4</td>
<td>-2.6 (-2.1 - -3.2)</td>
</tr>
<tr>
<td>Serum concentrations</td>
<td></td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.34 (2.26-2.47)</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (nmol/L)</td>
<td>43 (34-48)</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>4.3 (3.4-6.9)</td>
</tr>
</tbody>
</table>

Values are presented as median and range or number (n).

Glucose metabolism, body composition and testosterone levels
No changes in fasting glucose, glycated hemoglobin and fasting insulin levels were observed during the study period (Table 2). Glucose tolerance was assessed by an OGTT at the end of each intervention (PTH and control). Glucose and insulin values during OGTT were higher in two subjects and lower in the other two subjects after 12 weeks of PTH (1-34) treatment compared to no intervention (Figure 1A-B). In line, the area under the curve (AUC) and incremental AUC (iAUC) of glucose and insulin excursions after ingestion of glucose showed no consistent change after 12 weeks of PTH administration (Figure 1A-B). Hepatic insulin sensitivity and peripheral insulin sensitivity did not show a consistent change after 12 weeks of PTH (1-34) treatment compared to no intervention (Figure 2A-B). Moreover, no changes in body weight, fat mass, lean body mass, total body bone mineral content and total body bone mineral density were observed during the study period (Table 2). Despite the substantial difference in osteocalcin levels, testosterone showed a decrease in two of the subjects and an increase in the other subjects over time (Figure 3).
Figure 1 Glucose (mmol/L) (A) and insulin (pmol/L) (B) excursions following an oral glucose tolerance test (75 g of glucose) after 12 weeks of PTH (1-34) 20 μg/day (closed dots) and after 12 weeks of no intervention (open dots). Data are expressed as mean±SEM.
Figure 2 Hepatic insulin sensitivity (% suppression endogenous glucose production (EGP)) (A) and peripheral insulin sensitivity (rate of disappearance [Rd], μmol/kg·min) (B) after 12 weeks of no intervention (control) and 12 weeks of PTH (1-34) 20 μg/day.
Table 2 Metabolic parameters and bone turnover markers after 12 weeks of no intervention (control) and 12 weeks of PTH (1-34) 20 μg/day

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>62.0 (57.1-83.8)</td>
<td>63.1 (57.1-84.0)</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>26.2 (18.7-33.9)</td>
<td>27.1 (17.7-34.4)</td>
</tr>
<tr>
<td>Lean body mass (%)</td>
<td>70.8 (63.1-77.9)</td>
<td>69.9 (62.7-79.0)</td>
</tr>
<tr>
<td>DXA total body BMC (g)</td>
<td>2190.0 (1954.8-2453.7)</td>
<td>2230.4 (1895.5-2495.8)</td>
</tr>
<tr>
<td>DXA total body BMD (g/cm²)</td>
<td>1028 (1005-1117)</td>
<td>1054 (1009-1101)</td>
</tr>
<tr>
<td>DXA total body T-score</td>
<td>-1.8 (-2.1 - -0.80)</td>
<td>-1.6 (-2.0 - -1.0)</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>13.8 (11.3-23.6)</td>
<td>15.7 (10.3-21.0)</td>
</tr>
<tr>
<td>LH (U/L)</td>
<td>4.4 (3.5-5.3)</td>
<td>5.7 (4.3-6.3)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.7 (4.8-5.9)</td>
<td>5.6 (5.3-6.0)</td>
</tr>
<tr>
<td>Glycated haemoglobin (mmol/mol)</td>
<td>40 (39-41)</td>
<td>40 (38-41)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>45.5 (20.8-86.0)</td>
<td>50.5 (19.3-90.0)</td>
</tr>
<tr>
<td>CTx (ng/L)</td>
<td>342 (277-428)</td>
<td>436 (245-703)</td>
</tr>
<tr>
<td>P1NP (μg/L)</td>
<td>43 (33-61)</td>
<td>70 (46-111)</td>
</tr>
<tr>
<td>Osteocalcin (nmol/L)</td>
<td>2.1 (1.7-2.6)</td>
<td>4.8 (3.5-6.7)</td>
</tr>
</tbody>
</table>

Values are presented as median and range.

Figure 3 Plasma testosterone levels (nmol/L) after 12 weeks of no intervention (control) and 12 weeks of PTH (1-34) 20 μg/day.
Discussion

The preliminary results from this study demonstrate that daily PTH (1-34) administration for 12 weeks increases osteocalcin levels by 127%. These changes in osteocalcin levels were not associated with a consistent pattern of change in body composition, glucose tolerance, insulin sensitivity and testosterone levels.

From animal studies, it is well established that osteocalcin, especially in its undercarboxylated form, plays an important role in glucose homeostasis (3, 22, 23). These observations have led to several cross-sectional studies investigating the role of osteocalcin in glucose metabolism in humans. The results have been summarized in two recent reviews of the literature (6, 7). The majority of these studies is consistent with observations from animal models, reporting a negative correlation between serum levels of undercarboxylated or total osteocalcin and blood glucose, insulin resistance, diabetes, obesity or markers of the metabolic syndrome. This is contrary to the preliminary results presented in the present intervention study as we did not find any effect of marked elevations in osteocalcin levels by PTH on metabolic parameters. To date, only three studies have investigated the effects of interventions in bone metabolism affecting osteocalcin concentrations on glucose metabolism (8-10). In a study in 97 postmenopausal women, undercarboxylated osteocalcin levels increased in parallel with PTH (1-84) (n = 64) and decreased with bisphosphonate administration (n = 33). The three-month change in undercarboxylated osteocalcin in this study was inversely associated with body weight and positively associated with adiponectin at 12 months. However, three-month changes in undercarboxylated osteocalcin were not correlated with 12-month changes in insulin and glucose (9). In another study, significantly decreased levels of total and undercarboxylated osteocalcin were observed in 84 postmenopausal subjects following 16-weeks treatment with a bisphosphonate. After 16 weeks of treatment, fasting glucose levels increased significantly, but HOMA-IR increased significantly only in the subgroup with normal fasting glucose. No correlations were observed between changes in osteocalcin and changes in glucose parameters (8). Furthermore, inhibition of bone resorption in the FIT (alendronate), HORIZON-PFT (zoledronic acid) and FREEDOM (denosumab) trials was not associated with significant changes in fasting glucose levels or diabetes risk (10). In addition, two randomized trials involving the administration of vitamin K1, which is known to substantially lower undercarboxylated osteocalcin concentrations, did not observe an effect on fasting glucose and insulin resistance (24, 25). To summarize, evidence from cross-sectional studies points to a similar role for (undercarboxylated) osteocalcin in glucose metabolism in humans as has previously been observed in animals, but results from studies reporting interventions which affect osteocalcin concentrations are not unequivocal.

In addition to the metabolic phenotype, male osteocalcin knockout mice are characterized by a subfertile phenotype (1, 2). The endocrine role of osteocalcin in human gonadal function has currently been investigated only in a few cross-sectional studies (11-17). One large population-based study showed osteocalcin to be positively associated with total testosterone levels (11). Other studies did not find an association between osteocalcin and testosterone
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(12, 13, 17) or reported an association only in a selected population of young subjects (14) and subjects with metabolic disorders (15, 16). Our study is the first intervention study to investigate testosterone levels following increases in osteocalcin produced by PTH administration. In summary, the vast majority of cross-sectional studies, as well as this present study, do not support a biologically significant role for osteocalcin in the regulation of human testosterone levels, in contrast to animal models.

Evidence from longitudinal human studies suggests that (undercarboxylated) osteocalcin has little or even no effect on glucose homeostasis and testosterone levels. There are several explanations why mouse models do not translate to the human situation. Mice have three osteocalcin genes, whereas humans have only one (26). The human osteocalcin gene is upregulated by 1,25-dihydroxyvitamin D, whereas in mice opposite effects are seen (26). Furthermore, while osteocalcin is completely absent in osteocalcin knockout mouse models, this is not the case after antiresorptive therapies and vitamin K administration in humans although marked reductions in (undercarboxylated) osteocalcin levels are achieved.

Circulating osteocalcin exists in two forms, carboxylated on three glutamate residues, and undercarboxylated (27). In rodents, undercarboxylated osteocalcin has been identified as the biologically relevant form of osteocalcin (2, 3, 22). When interpreting the current literature it is important to know that different approaches to present results are chosen. Both direct (ELISA, ECLIA) and indirect (hydroxyapatite binding assay) methods to measure undercarboxylated osteocalcin are available (28, 29). In addition there are different ways to report undercarboxylated osteocalcin values, i.e. absolute values and percentages. The establishment of standardized procedures to measure and report total and undercarboxylated osteocalcin will resolve this issue.

In this study, we did not measure undercarboxylated osteocalcin, which in rodents has been regarded as the hormonally active form of osteocalcin (2, 3). However, total osteocalcin and undercarboxylated osteocalcin levels are highly correlated (14, 26, 30), making total osteocalcin levels a good indicator of undercarboxylated osteocalcin concentrations. Therefore, total osteocalcin can also be used as a biomarker when investigating the endocrine actions of osteocalcin.

There are several limitations to our study. First of all, this interim analysis was based on a very small study population. In addition, only male subjects above the age of 50 years with an indication for medical treatment of osteoporosis were included.

Since osteocalcin is not available for administration in humans, we used PTH treatment to increase endogenous osteocalcin levels. There are no studies available investigating the direct effects of PTH on insulin signaling and glucose homeostasis or plasma testosterone levels. To avoid potential confounding by PTH, future research should be invested in making (undercarboxylated) osteocalcin available for exogenous administration in humans.

The strength of this present study lies in its study design. By means of PTH administration a striking increase in endogenous osteocalcin levels was achieved. The increase was not significant due to our very small study population, but without exception osteocalcin levels
increased dramatically in all subjects. Due to the crossover design, paired comparison is possible in which subjects serve as their own control. In addition, glucose metabolism was investigated with a hyperinsulinemic euglycemic clamp, which is regarded as the gold standard for assessing insulin resistance.

Treatment with antiresorptive therapy for osteoporosis is associated with a decrease in (undercarboxylated) osteocalcin levels (9). Based on the currently available data there is no reason to assume that these patients are at increased risk for developing iatrogenic hypogonadism or diabetes mellitus and that caution is needed when prescribing these drugs. Unfortunately this also means that raising serum (undercarboxylated) osteocalcin levels carries no promise as an effective novel therapeutic approach in the prevention and treatment of diabetes mellitus or subfertility and gonadal dysfunction.

In conclusion, the preliminary results from this study do not support an endocrine role for osteocalcin in humans as has been observed in animal models. However, the sample size of the present intervention study is too small to draw definitive conclusions.
References


