Effect of intermittent PTH treatment on plasma glucose in osteoporosis: A randomized trial.

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Abstract
We investigated the effect of bone turnover on glucose homeostasis, fat distribution and adipokine production during anabolic treatment with PTH.
This is a parallel, randomized controlled, open label, trial. The randomization was done by computer generated tables to allocate treatments. Forty-six postmenopausal osteoporotic non-diabetic women were assigned to treatment with calcium and colecalcipherol with (24) or without (22) PTH 1–84. Patients were recalled after 3, 6, 12 and 18 months of treatment and markers of bone turnover, glucose metabolism, adipokine secretion and fat distribution were analyzed. Markers of bone turnover and adipokines were measured by ELISA. Glucose metabolism was evaluated by an oral glucose load test and insulin resistance and secretion were calculated. Fat and lean mass were evaluated by anthropometric measures. The effect of treatment on measured variables was analyzed by repeated measure test, and its effect on glucose was also evaluated by mediation analysis after correction for possible confounders. Twenty patients in the calcium and vitamin D groups and 19 in the group treated with PTH 1–84 completed the study. There were no significance adverse events.
Treatment with PTH increases osteocalcin, both total (OC) and undercarboxylated (uOC), and decreases blood glucose, without influence on insulin secretion, resistance and pancreatic β cell function. Treatment with PTH does not influence fat distribution and adipokine production. The results of the mediation analyses suggest a total effect of PTH on blood glucose, moderately mediated by OC and to a less extent by uOC.
Here we suggest that treatment with PTH influences glucose metabolism partially through its effect on bone turnover, without influence on insulin secretion, resistance, pancreatic β cell function and fat mass.

Abbreviations
OC, osteocalcin; uOC, undercarboxylated osteocalcin; iPTH, intermittent PTH; BAP, bone alkaline phosphatase; TRAP5b, Serum Tartrate Resistant Acid Phosphatase 5b; BMD, bone mineral density; OGTT, oral glucose tolerance test; ISOGTT, insulin sensitivity index; HOMA-IR, homeostasis model assessment of insulin resistance; FPG, fasting plasma glucose; FPI, fasting plasma insulin; IGI, insulinogenic index

Introduction
Glucose metabolism depends on a complex signal network that involves pancreatic islet cells, liver, fat, muscle, kidney and brain. In recent years the role of the skeleton in glucose and energy homeostasis has been studied. In particular the osteoblast-specific protein osteocalcin (OC), in its undercarboxylated form (uOC) appears to influence fat and glucose homeostasis in animal models. Mice knockout of both OC alleles had slightly increased fat mass and appear mildly hyperglycemic because of decreased β-cell proliferation, insulin secretion, and insulin resistance [1]. Conversely, the opposite phenotype null for the Esp gene, which encodes a tyrosine phosphatase that hampers glucose metabolism by inhibiting OC functions, had small fat pads, increased β-cell proliferation, enhanced insulin sensitivity, improved glucose tolerance and increased expression and serum levels of adiponectin. The mice with high levels of uOC did not become obese or glucose intolerant under conditions that would usually induce these metabolic abnormalities.
In vitro experiments showed that uOC induced adiponectin expression in cultured adipocytes; adiponectin acts like an insulin sensitizing adipokine. Administration of recombinant uOC to wild-type mice decreased fat mass, increased adiponectin expression, improved glucose handling, and attenuated weight gain and glucose intolerance in the setting of a high-fat diet [2].
An even more intimate relationship between skeleton and energy metabolism was demonstrated by recent genetic experiments that found that leptin, an adipocyte derived hormone, inhibits insulin secretion by decreasing the production of uOC and is also involved in osteoblast differentiation [3].
In human subjects, cross-sectional studies suggested an association between OC, glucose metabolism, and fat mass [4], [5], [6], [7], [8], [9] and [10]. Total serum OC was inversely associated with body fat, fasting glucose, and fasting insulin in older adults [5] and in obese children [11]. In patients affected by type 2

diabetes mellitus, uOC was inversely correlated with abdominal fat and with hemoglobin A1c [10].
The administration of intermittent subcutaneous PTH is approved for osteoporosis treatment and increases bone formation in humans [12] and [13]; it has been shown that treatment with PTH 1–34 in diabetic rats increased the serum OC levels and decreased the serum glucose levels without changing insulin levels [14].
In humans an interventional study suggested that early increase in uOC induced by treatment with PTH 1–84 is associated with reduction in body fat and glucose level after 12 months [15].
The aims of this study were to investigate the effect of treatment with PTH 1–84 on bone turnover, glucose homeostasis, fat distribution and adipokine production in non-diabetic osteoporotic patients.

Materials and methods

The study was approved by the Ethical Committee of our Hospital ("Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino - A.O. Ordine Mauriziano - A.S.L. TO1"). Each patient signed an informed consent prior to the recruitment.

Trial design

This is a parallel, randomized controlled, open label, trial (registered as PTH 1–84 EudraCT 2009-012397-12). The randomization was done by computer generated tables to allocate treatments.
Randomization was done by the principal investigator, patients were enrolled by participants in the study, and lab measurement and statistical analyses were done by those blind to treatment.

Participants

Forty-six women affected by postmenopausal osteoporosis followed at our hospital were enrolled in the study between January 2010 and March 2012. Patients affected by secondary osteoporosis, by diabetes or taking drugs active on bone, glucose or fat metabolism were not considered eligible for the study.
Patients were randomly assigned to treatment with calcium 1200 mg/day and colecalcipherol 800 UI/day with (24 patients, iPTH) or without (22 patients, controls) PTH 1–84 100 μg/day s.c. (Preotact®, kindly provided by Nycomed). This sample size provided an 80% power, assuming a two-sided significance level of 0.05, to detect differences in uOC greater than 1.71 (T-test on log-scale), considering previously reported median and interquartile ranges for uOC after iPTH treatment [15]. This effect is smaller than the one found in the randomized trial by Schafer et al. [15], in order to have enough power to focus also on the effect of uOC and OC on glucose metabolism. In the calcium and colecalcipherol treatment group 2 patients dropped out for adverse gastrointestinal events after the first 3 months of treatment, whereas in the PTH 1–84 there were 3 dropouts after the first month for low compliance to sub-cutaneous injection and 2 patients did not come back at 18 months visit for personal problems. Data from patients who dropped out within the first 3 months were not considered in the statistics, whereas data from patients who completed 12 months were included (Fig. 1).
The main outcome measures were markers of bone turnover, glucose metabolism, adipokine secretion and fat distribution. The measurements were done at baseline and after 3, 6, 12 and 18 months of treatment. Secondary outcome measure was evaluation of bone mineral density (BMD).
At baseline 25-OH vitamin D levels were measured by ELISA technique (DLD, Hamburg, Germany). Patients treated with iPTH get their injection at least 24 h before the blood exams in order to avoid the possible acute effect of PTH administration.

Bone turnover and bone density

As markers of bone formation we measured by ELISA technique: total OC (eBioscience, San Diego, CA), uOC (Takara, Shiga, JAP) and bone alkaline phosphatase (BAP, measured by QUIDEL kit, San Diego, CA).
Serum Tartrate Resistant Acid Phosphatase 5b (TRAP5b) was measured as marker of bone resorption by ELISA technique (QUIDEL, San Diego, CA). Markers of bone turnover were measured at enrollment and after 3, 6, 12 and 18 months of treatment, after overnight fasting.
The effect of treatment on BMD was assessed by bone densitometry on spine and femur performed at enrollment and after 18 months of treatment by Hologic QDR 4500 X-Ray densitometer.

Glucose metabolism

An oral glucose tolerance test (OGTT) with 75 g of glucose and blood sampling for glucose and insulin at 0 min, 30 min, 60 min, 90 min, and 120 min has been conducted at enrollment and after at 6, 12 and 18 months of therapy.
Insulin resistance was measured by Matsuda's insulin sensitivity index (ISOGTT) [16] and the homeostasis

model assessment of insulin resistance (HOMA-IR) [17]. ISOGTT was calculated as 10,000 / √ (FPG ∗ FPI) ∗ (G ∗ I), where FPG represents the fasting plasma glucose, FPI the fasting plasma insulin, G the mean plasma glucose during the OGTT and I the mean plasma insulin during the OGTT [16] HOMA-IR was calculated as FPG ∗ FPI / 22.5 [17].

Insulinogenic index (IGI) was calculated as [(30 min FPI − FPI) / (30 min G − FPG)] divided by the HOMA-IR (IGI/IR) [18].

Adipokine and fat distribution

In order to evaluate the possible effect of iPTH treatment on adipokine production we measured serum leptin and adiponectin by ELISA technique (R&D Duoset, Minneapolis, MN) at enrollment and after 3, 6, 12 and 18 months of treatment, after overnight fasting.

Body fat was assessed by plicometry (Mahr GMBH Esslingen) at each visit, and the Pollock, Schmidt and Jackson's formula on three sites (triceps, subscapular and abdomen) was applied to calculate fat percentage [19]. Fat distribution was also measured by the waist/hip ratio. Muscle mass was measured by brachial and calf circumferences.

In order to exclude the possible biases due to variation in caloric intake, dietetic intake was investigated through personal interview and caloric and nutrient intakes were calculated using the PROGEO software (Progeo S.r.l. Italy) at each visit.

The study flow chart is shown in Fig. 1.

Statistical analyses

The effect of treatment on markers of bone turnover, glucose metabolism parameters and adipokines was analyzed by repeated measure ANOVA. In order to evaluate the relationship between OC, uOC and FPG a linear regression model adjusted for treatment was carried out.

A mediation analysis was performed to evaluate if the effect of treatment on glucose level was mediated by OC level. Specifically we estimated separately:

i) the direct (unmediated) and indirect (mediated) effects of treatment on the glucose level at 6 months mediated by OC at 3 months

ii) the direct and indirect effects of treatment on the glucose level at 12 months mediated by OC at 6 months.

In order to obtain these estimates, two linear regression models on logarithmic scale were fitted, the first for glucose level dependent on treatment, OC level and their interaction and the second for OC level dependent on treatment; hence direct and indirect effects were estimated from the regression parameters in both models, provided certain identifiability assumptions and models are correctly specified [20]. A similar analysis was conducted by considering the association between glucose levels and uOC. The use of more sophisticated method to analyze repeated measures was not used because of low number of subjects included in this analysis.

The repeated measurements ANOVA testing the effect of treatment as well as the mediation analysis were adjusted for biomarkers that were unbalanced between the two treatment groups at baseline (uOC and TRAP5b). Statistical analysis was performed using statistical software STATA 11.1.

Results

There were no significant differences between the two treatment groups for the analyzed baseline variables with the exception of uOC and TRAP5b, that were higher in PTH treated patients (Table 1).

As expected the administration of iPTH increased markers of bone turnover, OC, BAP, TRAP5b (Figs. 2A–B–C) and uOC (Fig. 2D), whereas the treatment with calcium and vitamin D did not.

In secondary analyses, we found that iPTH increases lumbar BMD (0.710 ± 0.101 g/cm2 at baseline vs 0.784 ± 0.110 g/cm2 after 18 months $p < 0.001$), and total femur BMD (0.556 ± 0.089 g/cm2 at baseline vs 0.677 ± 0.075 g/cm2 after 18 months, $p = 0.001$), whereas there was weaker evidence on an effect of iPTH on femoral neck BMD (0.556 ± 0.084 g/cm2 at baseline vs 0.576 ± 0.098 g/cm2 after 18 months, p value = 0.071). Calcium and vitamin D treatment did not increase BMD in any of the analyzed sites.

Effect of iPTH on glucose level and mediation by OC and uOC

In patients treated with iPTH there was a decrease in FPG, the decrease was significant after 6 months of treatment and last for all the period analyzed, in patients treated with calcium and vitamin D alone there

was no decrease in FPG (Fig. 3A). Treatment with iPTH seems not to influence the other parameters of glucose metabolism analyzed, we found no variation in insulin secretion and resistance as measured by OGTT derived parameters (Figs. 3B, C, D).
Out of the 41 subjects, 3 patients had no data on OC and uOC at 3 and 12 months, whereas 4 patients (3 + 1) had no data on OC and uOC at 6 months. Hence linear regression models and mediation analyses were performed on patients having complete data on OC and uOC.
The linear regression analysis suggested that fasting plasma glucose is associated with OC (Table 2) and uOC mainly because of the treatment; only the association between uOC and FPG measured at 12 months was not influenced by treatment with iPTH (Table 2).
The mediation analysis suggested that the effect of iPTH on FPG at 6 months was moderately mediated by its effect on OC (45% of total effect), but not on uOC at 3 months (15% of total effect) (Table 3). However the effect of iPTH on FPG at 12 months was more markedly mediated by its effect on OC (62% of total effect) and uOC at 6 months (48% of total effect) (Table 3). Overall there was evidence of a total effect of iPTH on FPG, partially mediated by OC and to a less extent by uOC. If the interaction between treatment and OC/uOC levels was not taken into account, direct effects were emphasized compared to indirect
Effect of iPTH on body fat
Treatment with calcium and vitamin D with or without iPTH had no effect on total fat ($p = 0.528$), on waist/hip ratio ($p = 0.300$) and on muscle mass (arm, $p = 0.302$, calf, $p = 0.480$).
The fat mass did not correlate with bone turnover markers nor with uOC, whereas it correlates with leptin ($r = 0.67$, $p < 0.001$). The amount of leptin and adiponectin measured in the serum was not modified by treatment with calcium and vitamin D with or without iPTH (data not shown). Patients' dietary intake evaluated as total caloric intake and as macronutrients intake per kg of body weight was not influenced by treatment (data not shown).

Discussion
In recent years animal studies suggested that uOC influences glucose metabolism through multiple pathways: it increases insulin secretion [21] and [22], both directly and indirectly by increasing the gut secretion of glucagon-like peptide-1 [23]; moreover uOC promotes β-cell proliferation [2] and increases insulin sensitivity in peripheral tissues [1]. However, human studies found conflicting results: in some works uOC has been found to be related with fasting plasma glucose levels and to influence insulin sensitivity [24], [25] and [26], whereas other researchers found no association [27], [28] and [29]. The stronger association between insulin resistance and uOC in humans has been found in obese and/or diabetic patients [24], [25] and [26], whereas in lean subjects this relation is less evident [27]. The suppression of bone turnover with antiresorptive drugs in humans does not influence glucose metabolism [28] and [29], whereas the role of bone turnover in the control of glucose metabolism has been demonstrated in mice [30].
Here we studied if anabolic treatment with iPTH could influence glucose metabolism, fat amount and distribution and the production of adipocitokines by increasing bone turnover.
As expected iPTH significantly increases bone turnover along the 18 months of treatment, whereas calcium and vitamin D do not. Treatment with iPTH is effective in increasing the undercarboxylated fraction of OC as also shown by Schafer et al. [15]; the increase in uOC is evident after 3 months of treatment and last for all the treatment period. As demonstrated in animal studies OC and uOC affect glucose metabolism, hence a treatment able to influence these parameters may affect glucose homeostasis. Our results show that fasting plasma glucose is decreased in patients treated with iPTH, but not in patients treated with calcium and vitamin D alone. A previous study by Anastasilakis et al. [31] showed an adverse effect of treatment with teriparatide on glucose metabolism. In particular these authors showed an increased glucose and insulin level immediately after the injection of teriparatide and 6 months after treatment. To explain the differences between our result and this study, it is important to consider that Anastasilakis et al. evaluated the effect of the 1–34 fragment of PTH and not of the entire molecule as we did, the two molecules may have a different effect on glucose metabolism. Furthermore Anastasilakis et al. included in their study patients affected by diabetes or by impaired glucose tolerance, whereas we enrolled only non-diabetic patients. In another study from the same group the administration of teriparatide in non-diabetic patients did not affect glucose metabolism, whereas chronic hypersecretion in primary hyperparathyroidism increases insulin secretion after glucose oral load [32].

Schafer et al. [15] showed that the increase in uOC after 3 months of iPTH treatment correlates with the changes in body mass and adipokines after at 12 months, whereas Anastasilakis et al. [31] demonstrated a different effect of acute teriparatide administration with respect to chronic administration. Our study suggests that the increase in OC and uOC after 3 and 6 months of iPTH partially influences glucose measured at 12 months, this delayed effect is in line with the findings by Schafer et al. [15]. This observation allows us to hypothesize a metabolic control exerted on a long period more than an acute effect of iPTH, partially mediated by its effect on OC and uOC.

Literature data on the effect of uOC on insulin secretion are quite controversial, some animal and human studies show that uOC influences glucose levels without affecting insulin secretion and sensitivity [14] and [15], whereas others suggested an inverse relationship between uOC and insulin resistance [11], [33], [34], [35] and [36]. Some studies show that uOC influences β cell proliferation and activity [2], [21] and [22], whereas others do not [37].

A recent paper suggests that OC may be associated specifically with skeletal muscle insulin action, more than on its hepatic metabolism [38]; furthermore a study on patients affected by type 1 diabetes demonstrated that OC level is inversely correlated with glycemic control and BMI independently from residual pancreatic function [39]. The abovementioned studies could explain our data on the effect of iPTH on glucose metabolism partially mediated by osteocalcin, and to a less extent by uOC without influence on insulin secretion. This result is in contrast with animal data [1] and [2], whereas it confirms a human study by Díaz-López et al. [25]. In animal models uOC has a deeper effect on glucose metabolism with respect to OC [1] and [2], whereas in humans OC, but not uOC, was inversely associated with insulin resistance and with FPG [25].

In this study the higher effect of OC on glucose level could be due to the higher increase of this molecule following iPTH and to the low cohort size. The reduction in fasting glucose in our patients has no direct clinical effect, but this observation may be useful to confirm the role of the skeleton on glucose metabolism in humans.

The human studies on the effect of uOC on insulin resistance have been obtained mainly in diabetic subjects, it is well known that diabetes influences bone metabolism per se [40] and thus can alter the relationship between bone turnover and glucose metabolism; we enrolled in the study only lean women (mean BMI 23) without diabetes in order to exclude the influences of fat and high glucose levels on bone turnover and density.

In this study the mediation analysis suggests that iPTH has a direct effect on FPG beyond its effect on bone turnover, previous studies allow to hypothesize a direct effect of PTH on glucose metabolism as it has been suggested that continuous increase in PTH levels could impair glucose tolerance through: (i) increased intracellular free Ca concentration, which decreases insulin sensitivity by decreasing insulin-dependent glucose transport [38] and [41], (ii) decreased plasma phosphate levels which decrease insulin sensitivity, as insulin-dependent glucose uptake is closely related to phosphate uptake [42], and (iii) downregulation of glucose uptake following insulin stimulus [43]. All the abovementioned data suggested that continuous PTH secretion influences glucose metabolism by reducing insulin secretion whereas there are no previous experimental data on the effect of iPTH on glucose metabolism per se, without the mediation of bone anabolic effect.

Here we show no effect of iPTH on body fat amount and distribution, this result disagrees with the previous human study of Schafer et al. [15] that shows a decrease in body weight and fat mass after iPTH treatment; on the other hand a recent animal study by Hamann et al. [37] shows no effect of iPTH on rat body weight. Differently from Schafer et al. [15] we included in the study leaner patients (mean BMI was 22.7 in our study vs 25.3) this may influence the possible effect of iPTH on body weight, through its possible different effects on uOC. In fact a recent human study shows that the levels of uOC are influenced by weight, being lower in overweight and obese women [44]. Differently from Schafer et al. [15] we control the data on fat mass and distribution by an accurate dietary intake interview, this excludes any change in dietary habits during treatment.

We do not find any effect of iPTH on fat production of leptin and adiponectin whereas Schafer and colleagues [15] show a decrease in adiponectin after treatment with iPTH; this discrepancy could be explained by the lack of effect of iPTH on body weight and fat mass in our study. We also find no correlation between marker of bone turnover, fat amount and adipokines; previous literature found

conflicting results on this point: some studies suggested an inverse relationship [1], [6] and [10], whereas others did not find any correlation [40]. The direct correlation between leptin and fat mass in our study confirms the measure reliability.
The main limits of our study are the low sample size and the lack of more direct measure of insulin resistance as, for example, the glucose clamp technique. Moreover we do not include in the study a placebo controlled group, anyway it is unlikely that this methodological limitation can influence our results as in other human studies no placebo group has been included [15], [31] and [32]. The higher level of TRAP5b and uOC detected at basal level in the group treated with iPTH is presumably due to chance and to the small sample size, anyway this datum does not influence our results as shown by statistical analyses.

Conclusion

In conclusion here we show that iPTH decreases fasting glucose without affecting insulin resistance and secretion and has no effect on fat mass and distribution and on adipokine production. We also suggest that the iPTH effect on glucose metabolism is partially mediated through its effect on OC production.

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