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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 colecalciferol 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.

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].

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

☆ Trial registration EudraCT 2009-012397-12.

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In human subjects, cross-sectional studies suggested an association between OC, glucose metabolism, and fat mass [4–10]. 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 [12,13]; 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 groups 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 (IS_{OGTT}) [16] and the homeostasis model assessment of insulin resistance (HOMA-IR) [17]. IS_{OGTT} was calculated as $10{,}000 / \sqrt{(FPG * FPI) * (G * I)}$, 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 distributions

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.

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