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Differential impact of glucose administered intravenously or orally on bone turnover markers in healthy male subjects

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ABSTRACT

Background: Patients with type-1 (T1D) and type-2 diabetes mellitus (T2D) have an increased risk of hip fracture. The underlying mechanisms may involve disturbances in the incretin hormones. Our aim was to clarify if glucose administration i.e. orally or intravenously differentially affects bone turnover markers in healthy males.
Methods: 12 healthy males were included in a cross-over study consisting of three tests following an 8 hour fast. First, an oral glucose tolerance test (OGTT) was performed. Subsequently, we carried out an isoglycemic intravenous glucose infusion (IIGI) that closely mimicked the glucose response curve to the oral glucose load. We analyzed blood samples for the bone turnover markers serum C-terminal telopeptide of type I collagen (s-CTX) and serum procollagen type I N propeptide (s-P1NP), as well as insulin, glucose, gastric inhibitory peptide (GIP), glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2). Finally, eight of the twelve participants underwent a control experiment where they fasted for 3 h (Control).
Results: While OGTT induced a 50% reduction in s-CTX, only a ~30% reduction was seen during the IIGI and the Control. Neither intervention influenced s-P1NP. The concentration of insulin was highest during the OGTT. However, insulin was also increased significantly during the IIGI compared to the Control. Plasma concentrations of GIP, GLP-1 and GLP-2 were higher under the OGTT than during the IIGI and Control. A linear regression indicated that peak p-GIP significantly predicts nadir s-CTX ($p = 0.03$), and that peak p-GIP could explain 34% of the variability in nadir s-CTX (adjusted $R^2 = 0.34$).
Conclusion: This study indicates that glucose per se does not acutely affect bone turnover markers. However, gastrointestinal hormones, especially GIP, possibly in combination with hyperglycemia, may have an acute, uncoupling effect on bone turnover leading to a decrease in bone resorption but no change in bone formation.

1. Introduction

Type-1 (T1D) and type-2 diabetes mellitus (T2D) are associated with an increased risk of fractures [1]. A meta-analysis demonstrated, that T1D is associated with an increased risk of hip fracture of 6.94 fold (CI: 3.25–14.78) and T2D with an increased risk of 1.38 fold (95% CI: 1.25–1.53) compared to persons without diabetes [1]. Oddly, T2D subjects have higher bone mineral density (BMD) than healthy subjects [1]. Furthermore, the lower BMD among T1D subjects cannot explain the magnitude of the bone fracture incidence in this patient group [1]. The bone resorption marker C-terminal telopeptide of type I collagen (CTX) and the bone formation marker osteocalcin is decreased in patients with diabetes [2]. Subjects with diabetes mellitus (DM) have higher blood glucose levels than patients without DM. Therefore, increased circulating blood glucose may affect bone turnover and bone turnover markers. It is, however, unknown whether glucose per se has a negative effect on bones leaving them more prone to fracture, or whether other mediators are involved.

Glucose in a test meal or as an oral glucose tolerance test (OGTT) may potentially influence bone turnover. Thus, it has been shown that OGTT induces a 50% reduction in s-CTX over 2 h while an intravenous glucose tolerance test (IVGTT) induced less reduction in postmenopausal women [3]. There was only a <10% reduction in s-CTX during fasting [3]. The effect of an OGTT on s-CTX may be due to a direct effect of glucose, an effect of gastro-intestinal hormones or an additive or synergistic effect of glucose and gastro-intestinal hormones. The fact that an IVGTT induces less reduction in s-CTX than OGTT, indicates that gastrointestinal hormones may play an important role in the acute effect of

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glucose on bone resorption. However, the glucose infused during the IVGTT was given as a bolus infusion, so a difference in glucose curves between the OGTT and the IVGTT could be responsible for the differential effects [3]. Clowes et al. highlighted the importance of gastro-intestinal hormones as a mediator of the effect of glucose on bone. This was performed with octreotide, a somatostatin-analogue that blocks the release of gastrointestinal hormones. They demonstrated that octreotide decreased the reduction in s-CTX in response to an OGTT [4].

Our aim was to investigate the acute effects of glucose on bone markers per se in healthy males. We examined the acute effects of an oral glucose tolerance test (OGTT), an isoglycemic intravenous glucose infusion (IIGI) and a 3-hour fasting period on bone turnover markers and gastrointestinal hormones.

2. Methods

2.1. Registration and approval

The study was registered at ClinicalTrials.gov (NCT02213276). Approval was obtained from the Danish Data Protection Agency (2007-58-0010) and the Ethics Committee of the Central Denmark Region (1-16-02-377-13).

2.2. Study design and subjects

Twelve healthy Caucasian males, aged 20 to 50 years, were recruited by postings at Aarhus University and online at http://www.forsøgsperson.dk/. Subjects attended the hospital for an oral information session and signing a consent form. The study was conducted as a crossover study with two experiments on two separate days at least one week apart. After completing both experiments, participants were asked for participation in a control experiment. Eight of the twelve participants were enrolled in the control experiment. The day before each experiment, participants were asked to refrain from exercise, smoking and taking vitamin supplements. A standard meal delivered by the clinic was to be ingested between 17 and 23 o'clock and participants were asked to fast (water allowed) from 23 o'clock until they arrived for the experiment the next morning. They were asked to arrive by car or bus to the clinic.

2.3. Oral glucose tolerance test

The first experiment consisted of an OGTT where participants drank a glucose solution consisting of 82.5 g of glucose monohydrate (equal to 75 g of D-glucose), 225 ml of water and 225 mg of benzoic acid. Upon arrival of the participant, a peripheral intravenous catheter was placed in a cubital vein. Blood samples were collected at −15, −10, 0, 15, 30, 60, 120 and 180 min from ingestion of the glucose which lasted 5 min. Plasma glucose was measured at −15, −10 min from ingestion and every 5 min for the first 2 h and every 15 min for the last hour using an Accu-Chek Inform II apparatus (Roche Diagnostics, Basel, Switzerland). The apparatus is calibrated by the Department of Clinical Biochemistry, Aarhus University Hospital.

2.4. Isoglycemic intravenous glucose infusion

The second experiment consisted of an IGII where a glucose solution of 20% D-glucose was infused in a cubital vein to mimic the plasma glucose curve obtained during the OGTT. Upon arrival, two peripheral intravenous catheters were placed in contralateral cubital veins; one was used for glucose solution infusion and one for collecting blood samples. Blood samples were collected and plasma glucose measured at the same time points as for the OGTT. Infusion rate for the glucose solution was adjusted according to the measured actual plasma glucose level, the goal for the following measurement (the plasma glucose level measured at the OGTT for the same time point) and the anticipated insulin secretion rate of the participant.

2.5. 3-Hour fasting control

Upon arrival, a peripheral intravenous catheter was placed in a cubital vein. This marked the start of the control experiment. Blood samples were collected after 0, 1, 2 and 3 h. Plasma glucose was measured at the beginning of the experiment and after 1, 2 and 3 h using the Accu-Chek Inform II apparatus.

2.6. Blood samples

Before the first experiment, a fasting blood sample was drawn and plasma levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, HbA1c, TSH, PTH, vitamin D and ionized calcium were analyzed at the Department of Clinical Biochemistry at Aarhus University Hospital accredited according to ISO 15189. Blood samples for plasma analysis (insulin, GIP, GLP-1 and GLP-2) were centrifuged at 2000 $\times g$ for 15 min at 4 °C immediately after they were taken. Samples for serum analysis (CTX and P1NP) were kept at room temperature for 30–60 min and were then centrifuged at 2000 $\times g$ for 10 min. After this, all samples were kept at −80 °C until analysis.

2.7. Plasma analysis

Plasma insulin was measured by ELISA using a DAKO insulin kit (Code: K6219; Dako, Glostrup, Denmark). All samples were extracted in a final concentration of 70% ethanol before GIP and GLP-1 measurements and 75% before GLP-2 measurements. Total GIP was measured using a radioimmunoassay with a C-terminally directed antibody (code no. 80867), which reacts fully with intact GIP and N-terminally truncated forms as described by Lindgren et al. [5]. The standard was human GIP (Bachem, cat no. H-5645) and the tracer was ^{125}I-labeled human GIP (Perkin Elmer, cat no. Nex402). Total GLP-1 was measured as described by Ørskov et al. [6] using a radioimmunoassay (antibody code no 89390) specific for the C-terminal of the GLP-1 molecule and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. Intact GLP-2 was measured using a radioimmunoassay originally described by Hartmann et al. [7]. The antiserum (code no. 92160) is directed against the N-terminus of GLP-2 and therefore measures only fully processed GLP-2 of intestinal origin. For standards, we used recombinant human GLP-2 and the tracer was ^{125}I-labeled rat GLP-2 with an Asp33 → Tyr33 substitution. Sensitivity for all the radioimmunoassays was below 5 pmol/l, and intra assay coefficient of variation below 10%.

2.8. Bone turnover markers

The International Osteoporosis Foundation, and the International Federation of Clinical Chemistry and Laboratory Medicine recommend that serum procollagen type I N propeptide (s-P1NP), and serum C-terminal telopeptide of type I collagen (s-CTX) are used as reference bone turnover markers in clinical studies [8]. Serum CTX and P1NP were measured by immunometric sandwich assays using the COBAS 6000 E (Roche Diagnostics, Basel, Switzerland). The coefficient of variation for the analyses are 5% and 3.7%, respectively. Analyses were carried out at the Department of Clinical Biochemistry at Aarhus University Hospital.

2.9. Statistical analysis

Statistical analysis was carried out using the STATA 13 package (StataCorp, College Station, Texas, USA). Repeated measures ANOVA was performed to compare levels of glucose, bone turnover markers, insulin and gastrointestinal hormones between OGTT, IIGI and the 3-hour