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Effect of glucagon-like peptide-2 exposure on bone resorption: Effectiveness of high concentration versus prolonged exposure

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ABSTRACT

Objective: In healthy subjects, subcutaneous injections of GLP-2 have been shown to elicit dose-related decrease in the bone resorption marker, carboxy-terminal telopeptide of type I collagen (CTX), and have been proposed for the treatment of osteoporosis. This study investigated the relation between GLP-2 exposure and decreases in CTX in order to determine whether high concentrations or prolonged exposure was the most effective mode of administration. High GLP-2 concentrations resulted from iv bolus injections, whereas a more protracted stimulation was obtained by subcutaneous injections and the addition of an inhibitor of GLP-2 degradation, a DPP-4 inhibitor, sitagliptin.

Materials and methods: Eight healthy subjects were given: a) three intravenous injections of GLP-2 of 0.1, 0.4 and 0.8 nmol/kg, b) one subcutaneous injection of 1.6 mg GLP-2 and c) one subcutaneous injection of 1.6 mg GLP-2 preceded by an intake of sitagliptin. Blood was sampled for measurements of GLP-2 and p-CTX after each intervention.

Results: The 0.1, 0.4 and 0.8 nmol/kg GLP-2 injections dose-dependently elevated plasma GLP-2 concentrations and decreased CTX, but the decrease was similar regardless of dose. Subcutaneous GLP-2 caused a much more prolonged exposure (with a peak concentration corresponding to 0.4 nmol/kg IV) and was associated with a stronger and a more prolonged suppression of CTX, but in spite of significantly increasing exposure, the administration of sitagliptin, had no additional effect.

Conclusion: The high concentrations obtained by iv administration were less effective with respect to CTX suppression than the prolonged exposure (with much lower peak concentrations). GLP-2 agonists for osteoporosis treatment should therefore be long-acting for best efficacy.

1. Introduction and background

Bone tissue is constantly renewed and repaired by a coupled process of bone resorption and formation [1]. The bone resorption process can be assessed by measurements of the plasma concentration of carboxy-terminal telopeptide of type I collagen (p-CTX) [2]. p-CTX exhibits a diurnal variation with a peak during the night and a nadir late in the afternoon [3]. The daytime suppression of bone resorption appears to be related to ingestion of food [4], since ingestion of a mixed meal after fasting results in a significant reduction in the p-CTX concentration within 1–2 h [5], whereas fasting eliminates the circadian variation [3].

Intestinal glucagon-like peptide-2 (GLP-2) is produced in relation to meal ingestion in L-cells, a subset of enteroendocrine cells that are most abundant in the ileum and colon [6]. Shortly after meal initiation a rise in plasma GLP-2 concentrations is seen, and within 2–3 h it returns to basal levels [7,8].

Subcutaneous injections of GLP-2 have been shown to elicit a dose-related decrease in p-CTX in healthy subjects compared to baseline values [5], whereas bone formation as measured by serum-osteocalcin levels remains unaffected [9]. The mechanism is unclear, but it may be related to interactions with the enteric nervous system, in which there is an expression of the GLP-2 receptor. Since there apparently are no direct effects of GLP-2 on the bone cells and its actions involve complex indirect mechanisms, it is also not clear whether optimal suppression of bone resorption requires prolonged exposure of GLP-2 or high peak concentrations.

Abbreviations: GLP-2, glucagon-like peptide-2; p-CTX, plasma-carboxy-terminal telopeptide of type 1 collagen; AUC, area under the curve; pmol, picomole; nmol, nanomole; mg, milligram; DPP-4, dipeptidylpeptidase-4; cm, centimeter; kg/m^2, kilogram/square meter; EDTA, etylendiamintetraacetat; Asp, aspartic acid; Tyr, tyrosine; ELISA, enzyme-linked immunosorbent assay; iv, intravenous; sc, subcutaneous.

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GLP-2 is degraded through cleavage by the ubiquitously expressed proteolytic enzyme dipeptidyl peptidase-4 (DPP-4). The plasma half-life of intravenously infused GLP-2 is 7 min in healthy humans [10]. Blocking of DPP-4 degradation, either through Gly2 substitution, as in the Gly2-GLP-2 analog teduglutide, or through adjuvant use of DPP-4 inhibitors, extends the half-life of GLP-2 and confers greater biological potency [11–13].

In this study, we investigated the relationship between GLP-2 exposure and CTX-suppression. High peak concentrations were brought about by increasing doses of iv boluses of GLP-2, whereas prolonged exposure was obtained using sc injection, further augmented by the administration of a DPP-4 inhibitor.

2. Materials and methods

2.1. Participants

Eight healthy subjects (4 men, 4 women) aged 40.8 years (range 25–55 years) were recruited for the study. Their body height, weight and body mass index were 176.6 cm (range 161–188 cm), 74.4 kg (range 58.2–87.9 kg), and 23.8 kg/m^2 (range 20.9–27.4 kg/m^2), respectively. They all had an uneventful medical record, had no sense of physical or mental illness, and none were pregnant.

2.2. Experimental design

The subjects were instructed to fast from 10 pm the days before the scheduled 9 am visits the subsequent mornings at the Laboratory of the Department of Medical Gastroenterology, Rigshospitalet. The study consisted of five random, but not blinded or placebo controlled interventions, each lasting 3 to 6 h, with at least one-week in-between.

In random order, the healthy subjects received intravenous injections of native GLP-2, 0.1, 0.4 and 0.8 nmol per kilo body weight, given as a bolus. For these three interventions, the participants were provided with a large venous access (Venflon) in the left cubital vein for blood sampling. The GLP-2 bolus-injection was given intravenously at time 0 in the right arm followed by flushing with a few milliliters of isotonic sodium chloride. Blood (7 ml) was sampled at times −15, 0, 1, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min into EDTA containing vials. At a fourth intervention, the subjects were given 1.6 mg GLP-2 subcutaneously, and at a fifth intervention, 100 mg tablets of sitagliptin were given for the subjects to take orally at 10 pm the day before, and again 1 h before the intervention, followed by a subcutaneous 1.6 mg GLP-2 injection. At both subcutaneous interventions, the participants were provided with venous access in the left cubital vein for blood sampling. Blood was sampled at the following time points: −15, 0, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min. GLP-2 was injected subcutaneously at 0 min in the abdominal region. All blood samples were centrifuged to separate plasma within 30 min. Plasma was separated and stored at −20 °C until all samples were collected. Analyses were performed in one sequence.

2.3. Measurement of p-GLP-2 and of the biochemical marker of bone turnover p-CTX

2.3.1. GLP-2

Plasma GLP-2 was measured by radioimmunoassay with an N-terminal specific antiserum ID no. 92160, measuring only GLP-2 with an intact N-terminus, as described previously [6]. Synthetic human GLP-2 was used as the standard. The tracer was rat GLP-2 with an Asp^{33}→Tyr substitution, ^{125}I-labeled using the stoichiometric chloramine T method. This assay has a cross-reaction with synthetic human GLP-2 (3–33) of maximum 5.6% ± 1.8%. The experimental detection limit is 5 pmol/L, and the intra-assay coefficient of variation is 5% at a concentration of 40 pmol/L.

2.3.2. Plasma-CTX

Bone resorption was assessed from the concentration of p-CTX (Plasma CrossLaps One Step ELISA; Nordic Bioscience A/S, Denmark). The assay is a sandwich assay that detects collagen type I fragments generated during osteoclastic bone resorption and employs monoclonal antibodies recognizing C-telopeptide fragments of collagen type I a1 chains. The amount of bound complex is quantified by the use of a chromogenic peroxidase substrate. The intra-assay and inter-assay variations are 5.1% and 8.2%, respectively.

2.4. Statistical analysis

Results are expressed as mean ± SEM. GLP-2 increments and area under the curves, and CTX decreases in interventions 2, 3 and 5 were compared with the results in interventions 1 and 4, by using a non-parametric Mann–Whitney rank sum test at all sample times. A Friedman repeated measures analysis of variance on ranks was used to detect differences in the responses within the five interventions at the different sampling times, and a multiple pair wise procedure (Dunn's method) was used as the post hoc test to compare the responses at all sampling times in relation to the baseline values (−15 min). The SigmaStat version 3.1 was used for statistical calculations. A value of $p<0.05$ was considered significant.

2.5. Ethics

The Ethics Committee for Medical Research in Copenhagen approved the study protocol (H-A-2009-029), and the study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 1983. Informed consent was obtained from all participants before the study entry.

3. Results

3.1. Plasma GLP-2 profile after intravenous GLP-2 injections

GLP-2 injections of 0.1, 0.4, and 0.8 nmol/kg resulted in dose related increases in plasma GLP-2 concentrations from basal levels (~25 pmol/L) to mean peak concentrations of 1506, 5787, and 11,900 pmol/L respectively 1–2 min after injection (Fig. 1). After reaching peak concentrations plasma GLP-2 concentrations declined according to the half-life of the peptide, and reached basal levels of 90–180 min after injection.

Comparing the 0.1, 0.4 and 0.8 nmol/kg GLP-2 injections, the GLP-2 concentrations were significantly higher in the high dose compared to

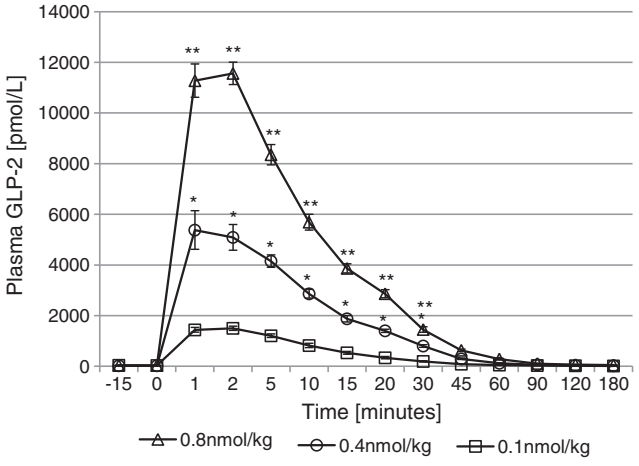

Fig. 1. Plasma GLP-2 concentration after iv injection. Mean plasma GLP-2 concentrations (pmol/L) after intravenous injections of 0.1, 0.4 and 0.8 nmol/kg GLP-2 in eight healthy subjects. *Significantly different from 0.1 nmol/kg and **significantly different from 0.1 and 0.4 nmol/kg. Data are presented as mean ± SEM.