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Acute peripheral administration of synthetic human GLP-1 (7–36 amide) decreases circulating IL-6 in obese patients with type 2 diabetes mellitus: A potential role for GLP-1 in modulation of the diabetic pro-inflammatory state?

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ABSTRACT

Background: To explore the effects of acute administration of GLP-1 and GIP on circulating levels of key adipocyte-derived hormones and gut-brain peptides with established roles in energy and appetite regulation, modulation of insulin sensitivity and inflammation.
Methods: Six obese male patients with diet-treated type 2 diabetes (T2DM) and 6 healthy lean subjects were studied. The protocol included 4 experiments for each participant that were carried out in randomised order and comprised: GLP-1 infusion at a rate of 1 pmol/kg/min for 4 h, GIP at a rate of 2 pmol/kg/min, GLP-1 + GIP and placebo infusion. Plasma leptin, adiponectin, IL-6, insulin, ghrelin and obestatin were measured at baseline, 15, 60, 120, 180 and 240 min following the start of infusion.
Results: Patients with T2DM had higher baseline IL-6 compared with healthy [day of placebo infusion: T2DM IL-6 mean (SEM) 1.3 (0.3) pg/ml vs 0.3 (0.1) pg/ml, $p = 0.003$]. GLP-1 infusion in T2DM was associated with a significant reduction in circulating IL-6 [baseline IL-6 1.2 pg/ml vs IL-6 = 0.7 at 120 min, $p = 0.0001$; vs IL-6 = 0.8 at 180 min, $p = 0.001$]. There was no significant change in leptin, adiponectin, ghrelin or obestatin compared to baseline on all 4 experimental days in both groups.
Conclusion: Short-term infusion of supraphysiological concentrations of GLP-1 in T2DM results in suppression of IL-6, a key inflammatory mediator strongly linked to development of obesity and T2DM-related insulin resistance. It remains to be confirmed whether GLP-1-based diabetes therapies can impact favourably on cardiovascular outcomes.

1. Introduction

In addition to the cardinal role of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) in the regulation of glucose homeostasis and the pathophysiology of type 2 diabetes mellitus (T2DM) [1,2], a number of other important extra-pancreatic effects have been recognised in recent years [3]. Receptors for GIP and GLP-1 have been shown to be expressed in adipose tissue. Studies in rodents support a role for endogenous incretin circuits in adipokine secretion and a regulatory role in adipocyte function [4]. Circulating adiponectin, resistin and plasminogen activator inhibitor-1 (PAI-1) levels after high fat feeding were found to be significantly increased in mice lacking either GIP or GLP-1 receptors, but double incretin receptor knockout mice failed to exhibit a rise in resistin and PAI-1 [4]. Acute and chronic administrations of GIP agonists in wild-type mice and mice lacking GLP-1 receptors are associated with a significant increase in plasma resistin levels, whereas no change was seen in GIP receptor$^{-/-}$ mice. These findings support the notion that GIP may be a potential regulator of adipokine secretion and that GIP signalling may represent an essential component of the adipocyte response to chronic nutritional excess [4].

Ghrelin, the most powerful peripherally circulating orexigen, is secreted by the X/A-like cells of the stomach. GLP-1 has potent anorexic properties and GLP-1 receptors are widely expressed on the stomach. One previous study in healthy volunteers has suggested that the anorexic effects of GLP-1 may be related to ghrelin suppression [5]. Obestatin is a peptide derived from the same gene as ghrelin that has shown in some studies to have opposing effects to ghrelin mainly with relation to food intake and gastric emptying. At present, the physiological importance of obestatin in humans remains controversial and its biological functions poorly understood [6].

We aimed to explore the effects in humans of the acute administration of synthetic human GLP-1(7–36 amide) and GIP, on the circulating levels of leptin and adiponectin (key adipocyte-derived hormones) and interleukin-6 (derived mainly from adipose tissue macrophages), with established roles in energy and appetite regulation, modulation of insulin sensitivity and inflammation. We also sought to determine the

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effects of the incretin hormones on the circulating levels of the appetite-regulating hormones ghrelin and obestatin.

2. Methods

2.1. Subjects

In order to minimise the effects of sex steroid fluctuations, only male subjects were invited for participation. The study involved the following two groups: 6 lean [body mass index (BMI) 20–25 kg/m^2] subjects with normal glucose tolerance and 6 obese (BMI > 30 kg/m^2) patients with T2DM on treatment with diet alone [7]. Participants were known to have had diabetes for at least 12 months with no history of ketosis or need for insulin. Exclusion criteria for the study included any regular drug treatment that could not be discontinued, any significant cardiovascular, pulmonary or gastrointestinal disease, history of ethanol dependence and presence of an eating disorder. In addition to the previous criteria, patients with diabetes were excluded if they had poor glycaemic control, defined as HbA1c > 64 mmol/mol (8.0%) for the purposes of this study. All eligible subjects provided informed written consent, and all studies were undertaken in accordance with the principles of the Declaration of Helsinki of the World Medical Association. The study protocol was granted ethical approval by the Liverpool Local Research Ethics Committee (project registration number REC: 04/Q1505/71).

2.2. Study protocol

At the baseline assessment visit, eligibility was confirmed, subjects provided informed written consent, and then they were weighed, their height was recorded, and waist circumference measured. Body composition was estimated by whole body bioelectrical impedance analysis (Tanita systems, Stokie, IL, USA). Each subject participated in four half-day studies: infusion of GLP-1 alone, GIP alone, GLP-1 and GIP together, and 10% glucose only (placebo). On each experimental day subjects attended in the morning at 0800 h after an overnight fast from 2200 h. Two venous cannulae were inserted, one in each forearm, one for administration of 10% glucose with or without GLP-1, GIP or GLP-1 + GIP and the other for venous blood sampling. Subjects and the investigator were blinded to the treatment given, which was allocated in random order. GLP-1 and/or GIP (1 pmol/kg/min and 2 pmol/kg/min in 1 l of 10% glucose respectively) or 10% glucose were infused intravenously from 08.30 am for 4 h. The infusion rates for GLP-1 and GIP were similar to the rates infused in similar sample size studies conducted over the last 20 years and have been shown to produce supra-physiological plasma concentrations of these two peptides [2,8–11]. Blood samples were taken at baseline, 15 min and then hourly up to 4 h following the commencement of the infusion. Samples were collected for later measurement of glucose, ghrelin, obestatin, insulin, leptin, adiponectin and interleukin-6 (IL-6). All samples were stored at −80 °C until assayed. Blood taken for these measurements was collected in tubes containing 50 μl aprotinin, to prevent degradation by DPP-IV and other proteolytic enzymes.

2.3. GLP-1 and GIP peptides

Synthetic human GLP-1 (7–36 amide) and GIP were obtained from Polypeptide Laboratories (Germany) and sterile-filtered and tested for stability by Stockport Pharmaceuticals (Stepping Hill Hospital, Stockport, UK). All vials with the peptides were securely stored at −80 °C until used for infusion.

2.4. Assays

Adiponectin, leptin and IL-6 were determined by commercial ELISAs (Oxford Biosystems, Oxford, UK). The inter-assay coefficient of variation (CV) for IL-6 was 4.4–5.4% and intra-assay CV 4.2–4.3%. The inter-assay CV for adiponectin was 5.7–6.7% for concentrations between 4.7 and 11.5 μg/ml and intra-assay CV 2.3–4.6% for concentrations between 5.8 and 14.3 μg/ml. The inter-assay CV for leptin was 10.2–12.7% for concentrations between 5.9 and 18.9 ng/ml and intra-assay CV 3.5–13.3% for concentrations between 1.5 and 43.4 ng/ml. Obestatin and ghrelin were measured with commercial EIA kits purchased from Bachem UK. Insulin was measured by an enzyme chemiluminiscent immunoassay (ECLIA) on a Roche Elecsys 2010 with an inter-assay CV between 3.5 and 4.1% for concentrations between 85 and 949 pmol/l.

2.5. Statistical analysis

Group by time interaction for IL-6, adiponectin, leptin, insulin, ghrelin and obestatin was analysed using ANOVA for multiple comparisons with baseline (Dunnett's method) for all four experimental days. Ghrelin, obestatin, leptin, adiponectin and IL-6 profiles during hormone infusions were compared with those observed during placebo infusion, within each group of subjects, using area-under-curve (AUC) analyses and paired *t*-test or Wilcoxon's sign rank test as appropriate. Areas under the curve for ghrelin, obestatin, leptin, adiponectin and IL-6 responses were calculated by trapezoidal integration. Data were analysed using SPSS version 18.0 (SPSS, Inc., Chicago, IL).

3. Results

The demographic and clinical characteristics of the participants in each group have been previously described [7] and are shown in detail in Table 1.

Glucose profiles in the two groups during the 4 experimental days are shown in Fig. 1. Patients with T2DM had higher baseline IL-6 compared with lean healthy [T2DM IL-6 mean (SEM) 1.3 (0.3) pg/ml vs 0.3 (0.1) pg/ml, $p = 0.003$, measurements obtained from experimental day with placebo infusion]. In patients with T2DM, GLP-1 infusion was associated with a significant reduction in circulating IL-6 (Fig. 2A) [baseline IL-6 1.2 pg/ml vs IL-6 = 0.7 at 120 min, $p = 0.0001$; vs IL-6 = 0.8 at 180 min, $p = 0.001$; vs IL-6 = 0.8 at 240 min, $p = 0.0007$, ANOVA for multiple comparisons with baseline, Dunnett's method], whereas in lean healthy subjects GLP-1 infusion had no effect on circulating IL-6 levels (Fig. 2A) [baseline IL-6 = 0.5 pg/ml vs IL-6 = 0.5 at 120 min, $p = 0.9$; vs IL-6 = 0.8 at 180 min, $p = 0.6$; vs IL-6 = 0.7 at 240 min, $p = 0.9$].

In patients with T2DM and in lean healthy subjects GIP infusion had no statistically significant effect on circulating IL-6 levels, although there was a trend towards higher levels during the period of infusion (Fig. 2B) [T2DM patients: baseline IL-6 = 1.1 pg/ml vs IL-6 = 1.5 at 120 min, $p = 0.6$; vs IL-6 = 1.7 at 180 min, $p = 0.3$; lean healthy subjects: baseline IL-6 = 0.3 pg/ml vs IL-6 = 0.4 at 120 min, $p = 0.9$ vs IL-6 = 0.9 at 180 min, $p = 0.5$].

In patients with T2DM, there was no significant change in circulating IL-6 compared to baseline during the experimental days when

Table 1
Demographic and clinical characteristics of the study participants [7].

Characteristic	Patients with T2DM	Healthy *p* value controls
Male sex (number)	6	6
Age (range)	51 (37–62)	39 (27–49) NS
BMI (kg/m^2)	41.7 (1.8)	24.9 (0.5) 0.0001
% body fat	38.6 (2.6)	19.7 (0.9) 0.0001
Waist circumference (cm)	127.3 (6.6)	91.5 (2.2) 0.0001
Duration of diabetes (years)	2.8 (0.3)	–
HbA1c (%)	6.5 (0.2) (48 mmol/mol)	–
Fasting plasma glucose (mmol/l)	7.4 (0.4)	5.1 (0.2) 0.002

Data are expressed as mean (SEM) unless otherwise indicated.
NS denotes non-significant.