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Glucose-dependent insulinotropic polypeptide lowers branched chain amino acids in hyperglycemic rats

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ABSTRACT

Hypersecretion of the incretin hormone glucose-dependent insulinotropic polypeptide (GIP) has been associated with obesity and glucose intolerance. This condition has been suggested to be linked to GIP resistance. Besides its insulinotropic effect, GIP also directly affects glucose uptake and lipid metabolism. This notwithstanding, effects of GIP on other circulating metabolites than glucose have not been thoroughly investigated. Here, we examined effects of infusion of various concentrations of GIP in normo- and hyperglycemic rats on serum metabolite profiles. We found that, despite a decrease in serum glucose levels (-26%, $p < 0.01$), the serum metabolite profile was largely unaffected by GIP infusion in normoglycemic rats. Interestingly, levels of branched chain amino acids and the ketone body β-hydroxybutyrate were decreased by 21% ($p < 0.05$) and 27% ($p < 0.001$), respectively, in hyperglycemic rats infused with 60 ng/ml GIP. Hence, our data suggest that GIP provokes a decrease in BCAA levels and ketone body production. Increased concentrations of these metabolites have been associated with obesity and T2D.

1. Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid hormone secreted from K-cells primarily in the duodenum and jejunum [1] in response to mainly lipids, amino acids [1] and glucose [2]. Recently, it has been established that GIP is also produced in pancreatic α-cells [3]. GIP is rapidly degraded by dipeptidyl peptidase 4 (DPP4) and its circulating half-life has been estimated to 7 min in humans and 2 min in rodents [4]. GIP is one of two known incretin hormones, the other being glucagon-like peptide-1 (GLP-1). The metabolic action of GIP is not only to potentiate glucose-stimulated insulin secretion [2], but also to increase glucose uptake [5] and lipid synthesis [6]. Genetic targeting of the GLP-1 receptor (GLP-1r) yields only modest effects on glycemia [7], due to up-regulation of GIP secretion and signaling [8]. Also, targeting of the GIP receptor (GIPr) yields modest effects on glycemia [9]. However, $GIPr^{-/-}$ mice are resistant to high-fat diet induced obesity [9]. Hence, GIP directly links over-nutrition to obesity and is therefore a potential target for anti-obesity drugs. Double incretin receptor knock-out mice, in which compensatory incretin action is inconceivable, possess a pronounced perturbation in oral glucose provoked insulin secretion and blood glucose [10].

Levels of GIP have been found to be elevated in obese and glucose intolerant subjects [11]. Recently, a variant in the *GIPr* gene was found to be associated with postprandial insulin levels, *via* reduced GIPr expression and GIP stimulated osteopontin expression [12]. More recently, the same variant was found to affect osteopontin expression and insulin resistance in adipose tissue [13].

Due to the localization of the K-cells in the gastrointestinal (GI) tract, bariatric surgery is expected to modulate secretion of GIP. Bariatric surgery, and especially Roux-en-Y gastric bypass, has been shown to improve glycemia within days after surgery, before onset of weight loss [14]. Two theories have been postulated for this effect. The hindgut theory implies that increased exposure of nutrients to the distal parts of the GI-tract increases secretion of incretins; especially GLP-1 secreted from L-cells [15]. The foregut theory implies that the exclusion of proximal sections of the GI-tract reduces secretion of anti-incretins and thereby potentially modulates secretion of incretins [16]. GIP, secreted from K-cells in the more proximal sections of the GI-tract, has thus been implicated as one of the hormones in the foregut theory [17]. However, studies on gastric bypass in humans have reported inconclusive results on changes in GIP levels [18,19]. Overall, previous studies suggest that GIP levels decrease after malabsorptive surgery and not after restrictive surgery [17].

Clearly, studies on the metabolic effects of GIP may provide novel knowledge on the pathogenesis of obesity and diabetes, two of the most common metabolic diseases in humans. Here, we report metabolic effects of GIP in rats infused with GIP at two different states of glycemia.

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Our results revealed that infusion of GIP provokes specific alterations in the metabolite profile and that these changes are more pronounced in hyperglycemic rats than in normoglycemic rats.

2. Materials and methods

2.1. Animals

Adult, male Sprague–Dawley rats (Taconic, Ry, Denmark), weighing approximately 300 g, with *ad libitum* access to pelleted food and tap water were used in the experiments. All experiments were approved by the local animal ethics committee at Uppsala University.

2.2. Research design

The rats were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (120 mg/kg body weight; Inactin™; Sigma-Aldrich, St. Louis, MO, USA). The animals were then placed on a servo-controlled heated operating table to maintain body temperature at 37.5–38 °C and breathed spontaneously through a tracheostomy. Heparinized catheters were inserted into the right carotid artery and left femoral vein. The tip of the former catheter was positioned in the ascending aorta 1–2 mm above the aortic valves and later used as an arterial access route. The catheter in the femoral artery was connected to a pressure transducer (PDCR 75/1; Druck Ltd., Groby, UK) to enable continuous measurement of mean arterial blood pressure, whereas the venous catheter was used to continuously infuse saline (6 ml/kg body weight/h) with 0.1% (w/v) bovine serum albumin (Sigma-Aldrich) added. When mean arterial blood pressure had remained stable for 15–20 min, GIP (Sigma-Aldrich) was added to the saline infusion at a dose of 0 ($n = 15$), 10 ($n = 7$), 20 ($n = 7$) or 60 ng/min ($n = 16$) for 30 min. 27 min after commencing the GIP-infusion, 1 ml of saline ($n = 45$) or 30% (wt/vol) D-glucose ($n = 44$) was injected intravenously. Three minutes after this, *i.e.* 30 min after initiating the GIP-infusion, the animals were exsanguinated by withdrawal of blood from the aorta. The blood was then centrifuged and serum was stored at −80 °C until analyzed.

2.3. Blood glucose and insulin measurements

Serum glucose and insulin were measured using the Freestyle Lite Blood Glucose Monitoring System (Abbott Laboratories, Abbott Park, IL) and a rat insulin ELISA from Mercodia (Uppsala, Sweden), respectively.

2.4. Metabolite profiling

Profiling of methoximated and trimethylsilylated metabolite derivatives was performed by gas chromatography/mass spectrometry (CG/MS) as previously described in detail [20]. Metabolites were extracted from 20 μl serum.

2.5. Data analysis and statistics

Metabolite data, expressed as the peak area of the corresponding mass chromatogram, were normalized to a set of stable isotope labeled internal standards [21], mean-centered, scaled to unit-variance and analyzed in Simca P + 12.0 (Umetrics, Umeå, Sweden). Orthogonal projections to latent structures (OPLS) models [22,23] were calculated to identify alterations in metabolite levels provoked by GIP-infusion, using the level of GIP as Y-variable, for both normoglycemic and hyperglycemic rats. OPLS removes systematic variation in the metabolite profiles that are orthogonal to the Y-variable, *i.e.* systematic variation in metabolite levels that is not related to the level of GIP infused. Loadings, scaled as correlations, from the two OPLS models were then combined in a shared and unique structures (SUS) [23] like plot to identify shared and unique alterations in metabolite levels provoked by GIP at normoglycemia and hyperglycemia. Hence, metabolites that correlate to GIP-infusion at normo- and/or hyperglycemia can be identified and visualized in only two dimensions. Boarders for unique and shared regions were derived from the loadings having a 95% confidence interval $\neq 0$ as estimated by jack-knifing. Hormone data were analyzed and results from the OPLS- and SUS-plots were verified by Student's t-test and one-way ANOVA followed by Tukey's test *post hoc* when more than two groups were compared. Data were expressed as fold to control (saline injections) ± SEM.

3. Results

3.1. Serum glucose and insulin

GIP-infusion dose-dependently increased insulin secretion in hyperglycemic rats (Fig. 1A). Insulin levels in hyperglycemic rats infused with saline were 12.4 ± 0.7 pg/l and increased to 20.7 ± 2.1 pg/l (1.7-fold, $p < 0.01$) and 44.8 pg/l (3.6-fold, $p < 0.001$) after infusion of 20 and 60 ng/ml GIP, respectively. Insulin levels were 2.3 ± 0.3 pg/l in normoglycemic rats infused with saline. Infusion of GIP did not affect levels of insulin in the absence of glucose infusion. Serum glucose levels in hyperglycemic rats infused with saline were 14.4 ± 0.5 mM. Levels of glucose were largely unaffected by GIP infusion in hyperglycemic rats, although a slight decrease (−10%, $p < 0.05$) was observed at 20 ng/ml GIP (Fig. 1B). Interestingly, infusion of GIP lowered glucose levels in normoglycemic rats; glucose levels were 21% lower (3.6 ± 0.1 mM; $p < 0.001$) in rats infused with 60 ng/ml GIP compared to rats infused with saline (4.6 ± 0.1 mM) (Fig. 1C). Glucose infusion alone increased levels of glucose 2.8-fold ($p < 0.001$) and levels of insulin 5.5-fold ($p < 0.001$).

3.2. Serum metabolite profiles

Next, alterations in levels of metabolites additional to glucose were investigated in normo- and hyperglycemic rats. Metabolite profiling yielded data on 70 metabolite derivatives, corresponding to 64 unique metabolites. Data covered major metabolite classes, including sugars, fatty acids, and amino acids. Overall, hyperglycemia provoked elevated levels of several amino acids; alanine (1.15-fold, $p < 0.05$), glycine (1.3-fold, $p < 0.05$), threonine (1.3-fold, $p < 0.05$), aspartate (1.4-fold, $p < 0.05$) and cysteine (1.2-fold, $p < 0.05$) were elevated at hyperglycemia, compared to normoglycemia in the absence of GIP-infusion. Levels of fatty acids trended towards a decrease; levels of myristate (−41%, $p = 0.085$), palmitate (−40%, $p = 0.068$), and oleate (−29%, $p = 0.075$) trended to be lower in hyperglycemic rats compared to normoglycemic rats in the absence of GIP-infusion.

Next, OPLS was used to relate the metabolite profiles of hyperglycemic rats to the amount of GIP infused. Thereby, systematic variation in metabolite levels provoked by GIP-infusion could be isolated from other types of variation [23]. The score scatter-plot, in which each data point represents a sample and its position is determined by levels of all detected metabolites, revealed a clear clustering of samples according to the GIP level (Fig. 2A). Hence, infusion of GIP provoked systematic alterations in the metabolite profiles in hyperglycemic rats. However, when the same model was applied to the normoglycemic rats, clustering was less clear (Fig. 2B). Thus, GIP-infusion yielded a weaker or less systematic shift in metabolism in normoglycemic rats. To unravel metabolites that underlie the clustering observed in the OPLS score-plots, the correlations from the two models were plotted in a SUS-like plot [23] (Fig. 2C). This plot revealed alterations in metabolite levels that were shared between normo- and hyperglycemia or unique to either of the glycemic states. Clearly, GIP-infusion was largely ineffective in modulating the metabolic response in normoglycemic rats. Decreased levels of phenylalanine were the only alteration shared between normo- and hyperglycemic rats. Raw data, expressed as normalized peak area (Fig. 3A), revealed that the levels of phenylalanine were