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Brief Communication

Attenuated secretion of glucose-dependent insulino-tropic polypeptide (GIP) does not alleviate hyperphagic obesity and insulin resistance in ob/ob mice

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ABSTRACT

Objective: Glucose-dependent insulino-tropic polypeptide (GIP) is released during meals and promotes nutrient uptake and storage. GIP receptor knockout mice are protected from diet induced weight gain and thus GIP antagonists have been proposed as a treatment for obesity. In this study, we assessed the role of GIP in hyperphagia induced obesity and metabolic abnormalities in leptin deficient (ob/ob) mice.

Methods: We crossbred GIP-GFP knock-in homozygous mice (GIP(gfp/gfp)) that have complete GIP knockout, and mice heterozygous for the ob mutation (Lepob/+) mice to generate Lepob/GIP(gfp/gfp), Lepob/ob/GIP(gfp/gfp), and Lepob/ob/GIP(gfp/gfp) mice. Male animals were weighed weekly and both oral glucose and insulin tolerance testing were performed to assess glucose homeostasis and circulating profiles of GIP and insulin. Body composition was evaluated by computerized tomography (CT) scan and analyses of indirect calorimetry and locomotor activity were performed.

Results: Postprandial GIP levels were markedly elevated in Lepob/ob/GIP(gfp/gfp) mice compared to Lepob/+/GIP(+/+) controls and were undetectable in Lepob/ob/GIP(gfp/gfp) mice. Insulin levels were equivalently elevated in both Lepob/ob/GIP(+/+) and Lepob/ob/GIP(gfp/gfp) mice compared to controls at 8 weeks of age but the hyperinsulinenia was marginally reduced in Lepob/ob/GIP(gfp/gfp) by 21 weeks, in association with amelioration of glucose intolerance. Both Lepob/ob/GIP(+/+) and Lepob/ob/GIP(gfp/gfp) mice remained equivalently insulin resistant. Body weight gain and subcutaneous and visceral fat volume of both Lepob/ob/GIP(+/+) and Lepob/ob/GIP(gfp/gfp) mice were significantly higher than that of Lepob/ob/GIP(+/+) mice, while no significant differences were seen between Lepob/ob/GIP(+/+) and Lepob/ob/GIP(gfp/gfp) mice. Locomotor activity and energy expenditure were decreased in both Lepob/ob/GIP(+/+) and Lepob/ob/GIP(gfp/gfp) mice compared to control Lepob/ob/GIP(+/+) mice, while no significant differences were seen between Lepob/ob/GIP(+/+) and Lepob/ob/GIP(gfp/gfp) mice. There was no significant difference in fat oxidation among the three groups. Fat content in liver was significantly lower in Lepob/ob/GIP(gfp/gfp) compared to Lepob/ob/GIP(+/+) mice, while that of control Lepob/ob/GIP(+/+) mice was the lowest.

Conclusions: Our results indicate that GIP knockout does not prevent excess weight gain and metabolic derangement in hyperphagic leptin deficient mice.

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Keywords GIP; Hyperphagia; Obesity; Insulin resistance; ob/ob

1. INTRODUCTION

Obesity is a significant global problem that considerably increases risk of cardiovascular disease, type 2 diabetes, insulin resistance and hyperlipidemia [1]. High caloric intake, whether from consumption of foods containing more fat or overeating, and inactive lifestyle, disrupt the balance between energy intake and output and worsen obesity [2–4]. Despite considerable efforts by both academia and industry, there are presently few effective treatment options for obesity. Given the role of the gut in the absorption of nutrients and production of potent regulatory peptides that coordinate nutrient disposal and satiety, it represents an important target organ for therapeutic strategies to combat both diabetes and obesity, particularly with members of the glucagon receptor family [5].

Ingested nutrients are sensed and absorbed by the intestine, triggering the release of hormones from enteroendocrine cells lining the gut epithelium. Two such hormones are the incretins gastric inhibitory polypeptide/glucose-dependent insulino-tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Mice with knockout of either the GIP receptor or GLP-1 receptor display glucose intolerance associated with...
2.2. Oral glucose tolerance tests (OGTTs) and insulin tolerance tests (ITTs)

OGTTs were performed with 8 and 21 week old mice after overnight fasting. Glucose (1 g/kg body weight) was administered by oral gavage and blood glucose levels were measured by glucometer (Sanwa Kagaku Kenkyusho, Nagoya, Japan). Plasma insulin and total GIP levels were measured at 0, 15, 30, 60, and 120 min after glucose administration. Plasma insulin levels were measured using a mouse insulin ELISA kit (Shibayagi, Gunma, Japan) and plasma total GIP levels were measured using a GIP ELISA kit (EMD Millipore Corporation, Billerica, MA, USA). ITTs were performed on mice at 10 and 30 weeks old using 1.0 U/kg and 3.0 U/kg human insulin (100 IU/ml, Eli Lilly, Hyogo, Japan), respectively, by intraperitoneal injection after 4–5 h fasting. Blood glucose levels were measured at 0, 30, 60, and 90 min after insulin administration.

2.3. Computerized tomography (CT) analysis

For CT analysis of body fat composition, mice were anesthetized at 36 weeks of age and scanned using a Latheta experimental animal CT system (LCT-100M, Aloka, Tokyo, Japan). Contiguous 2 mm slice images from shoulder to caudal region were used for quantitative analysis by Latheta software (version 3.00).

2.4. Indirect calorimetry and locomotor activity

Indirect calorimetry and locomotor activity of mice were measured at 34–35 weeks of age (ARCO 2000, ARCO System, Chiba, Japan). The mice were housed individually with free access to water and food. Energy expenditure (kcal/min/kg), fat oxidation (mg/min/kg), and locomotor activity (count/min) were measured every 5 min over 24 h.

2.5. Statistical analysis

Data represent mean ± SEM. Statistical differences between groups were assessed using one-way ANOVA with Tukey–Kramer Multiple–Comparison Test. P < 0.05 was considered statistically significant.

3. RESULTS

3.1. Body weight change, locomotor activity, energy expenditure, and fat oxidation of Lepob/+/GIP+/+, Lepob/ob/GIP+/+, and Lepob/ob/GIPgfp/gfp mice

Body weight of Lepob/+/GIP+/+, Lepob/ob/GIP+/+, and Lepob/ob/GIPgfp/gfp mice was tracked in cohort 1 and cohort 2 (Figure 1A). Body weight gain of Lepob/ob/GIP+/+ and Lepob/ob/GIPgfp/gfp mice was similar and significantly higher than that of Lepob/+/GIP+/+ mice (maximum weight: 68.0 ± 1.8 g, 70.9 ± 3.0 g, and 35.8 ± 0.8 g, respectively). Locomotor activity was similarly decreased in both the dark and light phases in Lepob/ob/GIP+/+ mice (52%, 44%) and Lepob/ob/GIPgfp/gfp mice (61%, 70%) compared to control Lepob/+/GIP+/+ mice (Figure 1B). Likewise, energy expenditure was decreased in Lepob/+/GIP+/+ mice (44%, 46%) and Lepob/ob/GIPgfp/gfp mice (49%, 48%) compared to control Lepob/+/GIP+/+ mice in dark and light phases, respectively, but did not differ between Lepob/ob mice with or without GIP (Figure 1B). There was no significant difference in fat oxidation among the three groups. There were no significant differences in food intake among the three groups (data not shown), and aside from the metabolic features indicated below, GIP knockout did not result in any other obvious phenotype or behavioral changes.
3.2. CT scan analysis of fat and liver in Lepob/+/GIP+/+, Lepob/ob+/GIP+/+, and Lepob/ob+/GIPgfp/gfp mice

Subcutaneous and visceral fat volumes were similarly increased in Lepob/ob+/GIP+/+ mice (≈13-fold, ≈10-fold) and in Lepob/ob+/GIPgfp/gfp mice (≈17-fold, ≈15-fold) relative to Lepob+/GIP+/+ mice (Figure 2A). Fat volumes were not significantly different between Lepob/ob+/GIP+/+ and Lepob/ob+/GIPgfp/gfp animals. Liver volume and fat content in liver were significantly increased in Lepob/ob+/GIP+/+ and Lepob/ob+/GIPgfp/gfp mice compared to Lepob+/GIP+/+ mice (Figure 2B). Fat content in liver was significantly lower in Lepob/ob+/GIPgfp/gfp mice compared to Lepob/ob+/GIP+/+ mice, although liver volume was not significantly different between the two groups (P = 0.05).

3.3. OGTTs and ITTs in Lepob+/GIP+/+, Lepob/ob+/GIP+/+, and Lepob/ob+/GIPgfp/gfp mice

During the OGTTs, plasma GIP levels and area under the curve (AUC) of GIP were significantly higher in Lepob/ob+/GIP+/+ mice than in Lepob+/GIP+/+ mice at both 8 weeks of age (peak values: 647 ± 42 vs. 42 C6 42 vs.
168 ± 32 pg/ml and 21 weeks of age (peak values: 902 ± 55 vs. 254 ± 19 pg/ml) (Figure 3A and B). As expected, GIP levels were below detection in Lep^{ob/ob}/GIP^{+/+}, Lep^{ab/ab}/GIP^{+/+}, and Lep^{ab/ab}/GIP^{gfp/gfp} mice at 8 weeks of age and both were significantly higher than in control Lep^{ob/ob}/GIP^{+/+} mice (Figure 3C). These findings paralleled plasma insulin levels, which were similarly increased in Lep^{ab/ab}/GIP^{+/+} and Lep^{ab/ab}/GIP^{gfp/gfp} mice relative to control Lep^{ob/ob}/GIP^{+/+} mice (Figure 3E). Blood glucose levels were significantly elevated in Lep^{ab/ab}/GIP^{gfp/gfp} mice relative to the others at 21 weeks old (Figure 3D), and this was associated with significantly lower plasma insulin levels at 0 and 15 min during the OGTT in Lep^{ab/ab}/GIP^{gfp/gfp} compared to Lep^{ab/ab}/GIP^{+/+} animals (Figure 3F). The AUC-insulin tended to be lower in Lep^{ab/ab}/GIP^{gfp/gfp} mice than in Lep^{ab/ab}/GIP^{+/+}, but it did not reach statistical significance (P = 0.08); insulin levels in Lep^{ab/ab}/GIP^{gfp/gfp} and Lep^{ab/ab}/GIP^{+/+} mice were markedly elevated compared to levels in Lep^{ob/ob}/GIP^{+/+} (AUC-insulin: 43-fold, 89-fold increase, respectively). During ITTs in 10 week old mice, blood glucose levels were not decreased in Lep^{ab/ab}/GIP^{+/+} and Lep^{ab/ab}/GIP^{gfp/gfp} animals, while blood glucose levels were reduced by 67% in Lep^{ab/ab}/GIP^{+/+} given the same 1 U/kg dose of insulin (Figure 3G). When given a higher dose of insulin (3 U/kg) at 30 weeks of age, blood glucose was maximally reduced by 35% similarly in both Lep^{ab/ab}/GIP^{+/+} and Lep^{ab/ab}/GIP^{+/+} mice (Figure 3H).
4. DISCUSSION

Currently, the only therapy leading to substantial and sustained body weight is bariatric surgery. These procedures can also produce a remarkable resolution of type 2 diabetes within days after surgery, long before any significant weight loss takes place, leading some to perform bariatric surgery to treat diabetes even in non-obese individuals [26–28]. The altered flow of nutrients in the gut following bariatric surgery may be associated with adaptive changes in the enteroendocrine cell populations [29–31] and altered production of gastrointestinal hormones, including increases in plasma GIP and GLP-1 levels post surgery [28,32,33]. Changes in basal and/or postprandial release of gut hormones are among the potential mechanisms of improved glucose homeostasis and weight loss following bariatric surgery [34,35]. Therefore, it may be possible to mimic the effects of surgery by gut hormone delivery and single-molecule peptides integrating the complementary actions of multiple hormones have demonstrated promising results [5]. A unimolecular dual incretin derived from intermixed sequences of GLP-1 and GIP demonstrated enhanced anti-hyperglycemic efficacy relative to selective GLP-1 agonists in rodents, monkeys, and humans [36]. Furthermore, while a selective GIP agonist did not alter body weight in high fat fed mice, the co-agonist treatment produced significant weight loss [36]. Even greater efficacy was obtained in high fat fed mice with a triagonist incorporating a glucagon sequence for the synergistic action of glucagon to increase energy expenditure [37]. Therefore, activation of GIP receptors could be part of an effective strategy to treat diabetes and obesity.

Shortly after its discovery, endogenous GIP was implicated in linking over-nutrition to the development of obesity [38,39], in part because the expression of GIP appears to be coordinated with nutritional status. Oral fat is a potent stimulator of GIP release that is augmented by bile, and mediated through direct actions on K-cells via fatty acid-binding protein 5 and G protein-coupled receptor 120 [40,41]. Diets rich in fat increase intestinal K-cell number [42], GIP expression and circulating GIP levels [43], and the GIP response to oral glucose is enhanced by prior exposure to a high-fat diet [44]. Obese individuals have elevated plasma GIP levels that are associated with reduced postprandial plasma triglycerides, suggesting a role for GIP in triglyceride uptake [39,45]. GIP reduces plasma triglyceride increments following meals [46], an effect that could be mediated in part by increasing the activity of lipoprotein lipase, by direct actions of GIP on adipocytes [47,48]. In some rodent models of obesity, the insulinotropic action of GIP is no longer restrained at basal glucose levels and thus can contribute to hyperinsulinemia [44]. We observed marked elevations of both GIP and insulin in Lep<sup>db/db</sup> mice and perhaps their combined anabolic activity contributed to the excessive fat mass. Consistent with a role of GIP in fat accumulation, GIP receptor knockout mice were protected from weight gain and hepatic steatosis when placed on a high fat diet [12,49]. Moreover, when crossed onto mice with the <i>ob</i> mutation, the severity of obesity in homozygous offspring was reduced by 23%, although these mice remained almost twice the weight of control mice [12]. These findings contrast our observations in which complete ablation of GIP in homozygous <i>db/db</i> mice had no impact on weight gain, while hepatic fat content was modestly reduced. It is difficult to reconcile these differences resulting from knockout of GIP versus its receptor, particularly as alternate endogenous ligands for the GIP receptor have not been reported. Perhaps variations in diets, housing conditions or mouse microbiomes contributed to the differences. In our studies, Lep<sup>db/db</sup>/GIP<sup>fl/fl</sup> mice had insulin levels equivalent to Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice at 8 weeks of age and lower insulin levels at 21 weeks, yet they still remained severely hyperinsulinemic. In contrast, we previously observed a complete normalization of insulin levels in GIP knockout mice on high fat diet, associated with a significant reduction in weight gain relative to wild type controls [21]. A reduction in insulin production has been demonstrated to dramatically reduce weight gain in both <i>ob/ob</i> mice [50] and mice on a high fat diet [51]. We speculate that the reduction in insulin achieved in the Lep<sup>ob/ob</sup>/GIP<sup>fl/fl</sup> animals in our current study was insufficient to promote weight loss.

It is possible that regulation of adiposity and glucose homeostasis by GIP are in part mediated by altering leptin levels and/or leptin signaling. However, we are unaware of reports that support this mechanism of action of GIP. In addition, leptin levels in GIP receptor knockout mice [12,52] and mice with ablation of K-cells [20] remained proportional to fat mass, suggesting that GIP action does not directly regulate leptin production. The concept of an adipobenteroendocrine axis has been proposed, based upon observations that leptin directly stimulates GLP-1 secretion from rodent and human intestinal L cells [53], but whether leptin regulates GIP secretion from K-cells is unknown. Our mouse model enabled us to investigate the impact of GIP deficiency independent of leptin signaling. Collectively, our findings suggest that endogenous GIP is not involved in the development of obesity in mice with complete absence of leptin.

DISCLOSURE STATEMENT

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CONFLICT OF INTEREST

N. Inagaki served as a medical advisor for Takeda, Taisho Pharmaceutical, GlaxoSmithKline, and Mitsubishi Tanabe Pharma, and lectured for MSD, Sanofi, Novartis Pharma, Dainippon Sumitomo Pharma, Kyowa Kirin, and Mitsubishi Tanabe Pharma and received payment for services. No other potential conflicts of interest relevant to this article are reported.

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