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

Author(s): Shimazu-Kuwahara, Satoko; Harada, Norio; Yamane, Shunsuke; Joo, Erina; Sankoda, Akiko; Kieffer, Timothy J.; Inagaki, Nobuya

Citation: Molecular Metabolism (2017), 6(3): 288-294

Issue Date: 2017-03

URL: http://hdl.handle.net/2433/218733

Right: © 2017 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Type: Journal Article

Textversion: publisher

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

Satoko Shimazu-Kuwahara 1, Norio Harada 1, Shunsuke Yamane 1, Erina Joo 1, Akiko Sankoda 1, Timothy J. Kieffer 2, Nobuya Inagaki 1, * 

ABSTRACT

Objective: Glucose-dependent insulinotropic 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 (Lepob/ob) mice.

Methods: We crossed bred GIP-GFP knock-in homozygous mice (GIPgfp/gfp) that have complete GIP knockout, and mice heterozygous for the ob mutation (Lepob/+ ) mice to generate Lepob/+ /GIPgfp/gfp, Lepob/+ /GIP+/+, and Lepob/ob/GIPgfp/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/GIPgfp/gfp mice compared to Lepob/+ /GIP+/+ controls and were undetectable in Lepob/ob/GIP+/+ mice. Insulin levels were equivalently elevated in both Lepob/ob/GIPgfp/gfp and Lepob/ob/GIP+/+ mice compared to controls at 8 weeks of age but the hyperinsulinemia was marginally reduced in Lepob/ob/GIPgfp/gfp mice by 21 weeks, in association with amelioration of glucose intolerance. Both Lepob/ob/GIPgfp/gfp and Lepob/ob/GIP+/+ mice remained equivalently insulin resistant. Body weight gain and subcutaneous and visceral fat volume of both Lepob/ob/GIP+/+ and Lepob/ob/GIPgfp/gfp mice were significantly higher than that of Lepob/+ /GIP+/+ mice, while no significant differences were seen between Lepob/ob/GIP+/+ and Lepob/ob/GIPgfp/gfp mice. Locomotor activity and energy expenditure were decreased in both Lepob/ob/GIP+/+ and Lepob/ob/GIPgfp/gfp mice compared to control Lepob/+ /GIP+/+ mice, while no significant differences were seen between Lepob/ob/GIP+/+ and Lepob/ob/GIPgfp/gfp mice. There was no significant difference in fat oxidation among the three groups. Fat content in liver was significantly lower in Lepob/ob/GIPgfp/gfp compared to Lepob/ob/GIP+/+ mice, while that of control Lepob/+ /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.

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 potential 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 insulinotropic 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

1Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan
2Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada

* Corresponding author. Fax: +81 75 771 6601. E-mail: inagaki@kuhp.kyoto-u.ac.jp (N. Inagaki).

Abbreviations: GIPR, GIP receptor; GFP, green fluorescent protein; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; CT, computerized tomography

Received December 26, 2016 • Revision received January 11, 2017 • Accepted January 16, 2017 • Available online 19 January 2017

http://dx.doi.org/10.1016/j.molmet.2017.01.006
blunted insulin secretion [6,7]. The ability of these hormones to act directly on β-cells to augment insulin secretion during meals in a glucose-dependent manner is the basis for the treatment of diabetes by incretin mimics and inhibitors of the enzyme dipeptidyl-peptidase 4 (DPP-4) that rapidly degrades GIP and GLP-1 [8].

GIP is secreted by K-cells during meals, particularly when the cells come in contact with fat or glucose [9]. In addition to stimulating brisk release of insulin [10], GIP promotes adipogenesis and lipid accumulation in adipocytes [11,12]. Therefore, there has been some debate over whether GIP agonists used to improve glucose homeostasis may increase adiposity while GIP antagonists could promote weight loss [13]. Several studies have convincingly demonstrated glucose lowering effects following long-term administration of DPP-4 resistant GIP analogs in rodents [14–17]. Moreover, somewhat surprisingly, chronic overexpression of GIP in mice reduced diet-induced obesity and steatosis, in addition to improving glucose homeostasis [18]. Yet a seminal study revealed that mice with knockout of the GIP receptor are protected from high fat diet induced obesity and the development of insulin resistance [12]. Moreover, inhibition of GIP signaling in this model increases fat oxidation in peripheral tissues in association with increased adiponectin levels [19]. These findings are bolstered by studies demonstrating that reducing circulating GIP levels with K-cell ablation [20], disruption of GIP gene expression [21], immunoneutralization [22], or vaccination against GIP [23] also reduced weight gain in mice following high fat diet, without impairing glucose homeostasis.

Here, we examined whether complete ablation of GIP production could reduce weight gain in the absence of the adipocyte hormone leptin, a condition that results in extreme hyperphagia, obesity, hyperinsulinemia, and insulin resistance, in both mice [24] and humans [25]. We found that Lep<sup>ob/ob</sup> mice became equally obese and insulin resistant whether or not GIP was present, suggesting that GIP antagonism is unlikely to be effective at improving metabolism in extreme obesity associated with defective leptin action.

2. MATERIALS AND METHODS

2.1. Animals

The insertion of a sequence encoding green fluorescent protein (GFP) into preproGIP gene disrupts the expression of GIP, resulting in complete GIP peptide knockout in homozygous animals; GIP-GFP knock-in (GIP-GFP) mice were generated as described previously [21]. Leptin-knockout heterozygous (Lep<sup>ob/+</sup>) mice were purchased from Charles River Laboratories, Inc., Kanagawa, Japan. We first crossedbred Lep<sup>ob/+</sup> mice and GIP-GFP homozygous (GIP<sup>gfp/gfp</sup>) mice and generated Lep<sup>ob/+</sup>/GIP<sup>gfp/gfp</sup> mice. Next, we crossedbred Lep<sup>ob/+</sup>/GIP<sup>gfp/gfp</sup> mice to each other to produce Lep<sup>ob/+</sup>/GIP<sup>gfp/gfp</sup>, Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>, and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice. All experiments were conducted with two cohorts of male mice; oral glucose tolerance tests (OGTTs) and insulin tolerance tests (ITTs) were performed on cohort 1, and computerized tomography (CT) analysis, indirect calorimetry and locomotor activity were performed with cohort 2. The mice were housed in groups (n = 4 for Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>, n = 6 for Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>) at 25 °C with a 10:14-h dark–light cycle under free access to water and food.

3. RESULTS

3.1. Body weight change, locomotor activity, energy expenditure, and fat oxidation of Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>, Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>, and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice

Body weight of Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>, Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup>, and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice was tracked in cohort 1 and cohort 2 (Figure 1A). Body weight gain of Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice was similar and significantly higher than that of Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice (maximum weight: 68.0 ± 1.8 g, 70.9 ± 3.0 g, 35.8 ± 0.8 g, respectively).

Locomotor activity was similarly decreased in both the dark and light phases in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice (52%, 44%) and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice (61%, 70%) compared to control Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice (Figure 1B). Likewise, energy expenditure was decreased in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice (44%, 46%) and Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice (49%, 48%) compared to control Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice in dark and light phases, respectively, but did not differ between Lep<sup>ob/ob</sup> 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/þ/GIPþ/þ, Lepob/ob/GIPþ/þ, and Lepob/ob/GIPgfp/gfp mice

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

3.3. OGTTs and ITTs in Lepob/þ/GIPþ/þ, Lepob/ob/GIPþ/þ, 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/GIPþ/þ mice than in Lepob/þ/GIPþ/þ mice at both 8 weeks of age (peak values: 647 ± 42 vs. 42 ± 10 ng/ml).
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<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice. Blood glucose levels and glucose AUC were similar in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice at 8 weeks of age and both were significantly higher than in control Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice (Figure 3C). These findings paralleled plasma insulin levels, which were similarly increased in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice relative to control Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice (Figure 3E). Blood glucose levels were significantly elevated in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> 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<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> compared to Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> animals (Figure 3F). The AUC-insulin tended to be lower in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> mice than in Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup>, but it did not reach statistical significance (P = 0.08); insulin levels in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice were markedly elevated compared to levels in Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> mice (AUC-insulin: 43-fold, 89-fold increase, respectively). During ITTs in 10 week old mice, blood glucose levels were not decreased in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> animals, while blood glucose levels were reduced by 67% in Lep<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> 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<sup>ob/ob</sup>/GIP<sup>gfp/gfp</sup> and Lep<sup>ob/ob</sup>/GIP<sup>+/+</sup> 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 tritagonist 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 Lepob/ob 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 ob 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 ob/ob 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, Lepob/ob/GIPfl/fl mice had insulin levels equivalent to Lepob/ob/GIP+/+ 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 ob/ob mice [50] and mice on a high fat diet [51]. We speculate that the reduction in insulin achieved in the Lepob/ob/GIPfl/fl 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 adipo–enteroendocrine 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

This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEEXT), Japan Society for the Promotion of Science (JSPS), Ministry of Health, Labour, and Welfare, Ministry of Agriculture, Forestry and Fisheries, Japan Diabetes Foundation, Japan Association for Diabetes Education and Care, Merck Sharp & Dohme (MSD) Life Science Foundation, and Public Interest Incorporated Foundation, Japan Diabetes Foundation, Suzuken Memorial Foundation.

ACKNOWLEDGMENTS

The authors thank Mr. Shoichi Asano and Dr. Daniela Nasteska from the Department of Diabetes, Endocrinology and Nutrition, Graduate School of medicine, Kyoto University, for technical support regarding the study. T.J. Kieffer gratefully acknowledges fellowship support from JSPS while on sabbatical at Kyoto University.

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.

REFERENCES


