Pancreatic and Extrapancreatic Effects of Gastric Inhibitory Polypeptide

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The hormonal factor(s) implicated as transmitters of signals from the gut to pancreatic β-cells is referred to as incretin, and gastric inhibitory polypeptide (GIP) is identified as one of the incretins. GIP is a gastrointestinal peptide hormone of 42 amino acids that is released from duodenal endocrine K-cells after absorption of glucose or fat and exerts its effects by binding to its specific receptor, the GIP receptor. By generating and characterizing mice with a targeted mutation of the GIP receptor gene, we have shown that GIP has not only an insulinotropic role, but also physiological roles on fat accumulation into adipose tissues and calcium accumulation into bone. We here propose a new acronym, GIP, for gut-derived nutrient-intake polypeptide. *Diabetes* 55 (Suppl. 2):S86–S91, 2006

GASTRIC INHIBITORY POLYPEPTIDE AND GASTRIC INHIBITORY POLYPEPTIDE RECEPTOR

Gastric inhibitory polypeptide (GIP), also designated as glucose-dependent insulinotropic polypeptide, is a peptide hormone of 42 amino acid residues (1), posttranslationally processed from a precursor, preproGIP, of 153 amino acid residues (2). GIP is a member of a family of structurally related hormones that includes secretin, glucagon, and vasoactive intestinal peptide. The GIP moiety is flanked by a signal peptide of 21 residues and a peptide of 30 amino acids, and a peptide of 60 amino acids at its NH_2- and COOH-termini, respectively (Fig. 1*A*). Prohormone convertase 1/3 is essential and sufficient for endoproteolytic processing to produce mature GIP (3). GIP is secreted from specific endocrine cells (K-cells), which are scattered in the epithelium of the upper part of small intestine (4) after ingestion of a meal (5). Once released, GIP is subjected to NH_2-terminal degradation by dipeptidyl peptidase-IV (DPP-IV), yielding GIP (3-42) as the primary metabolite (6,7), which acts as a GIP receptor antagonist (8).

GIP exerts its effects by binding to its specific receptor, GIP receptor, activating adenylyl cyclase and increasing intracellular cAMP concentrations (9–11). The GIP receptor has seven potential membrane-spanning domains, a feature characteristic of the secretin/glucagon/vasoactive intestinal peptide receptor family of G protein–coupled receptors (Fig. 1*B*).

GIP AS AN INSULINOTROPIC FACTOR

Type 2 diabetes is characterized by various degrees of insulin resistance and pancreatic β-cell dysfunction. As liver, skeletal muscle, and adipose tissues become increasingly resistant to the action of insulin, the compensatory insulin secretion from pancreatic β-cells becomes insufficient to maintain blood glucose levels within the normal physiological range, leading to high blood glucose levels (12).

Pancreatic β-cell dysfunction is characterized by impaired insulin secretion in response to glucose. Physiologically, a much greater insulin response is observed after oral glucose loading than after intravenous injection of glucose. Elevation of plasma glucose levels directly stimulates insulin secretion from pancreatic β-cells, through glycolysis, mitochondrial oxidation, elevation of intracellular ATP-to-ADP ratio, closure of ATP-sensitive potassium channel, opening of voltage-dependent calcium channel, and elevation of intracellular calcium ion concentration. In addition to the above-mentioned glucose-induced insulin secretion, transmitters of signals from the gut to pancreatic β-cells are assumed to be responsible for greater insulin response after oral glucose loading. The hormonal factor(s) implicated as transmitters of signals

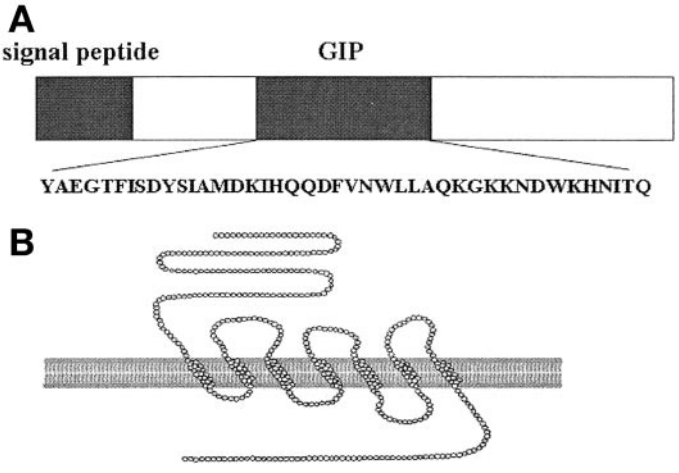

FIG. 1. Structure of GIP and GIP receptor. *A*: Human GIP is processed from a precursor, preproGIP (2). Amino acid residues are expressed in a single letter. *B*: Predicted secondary structure of human GIP receptor (11).

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DPP-IV, dipeptidyl peptidase-IV; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1.

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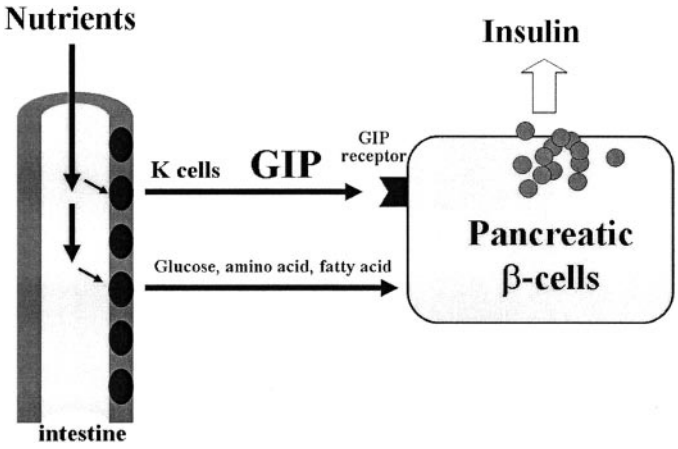

FIG. 2. GIP as incretin. GIP is released from small intestine after ingestion of a meal and stimulates insulin secretion from pancreatic β-cells.

from the gut to pancreatic β-cells was referred to as incretin (13,14).

Incretin is characterized by its release from gut after ingestion of a meal and the stimulatory effects on insulin secretion. In vitro studies using perfused pancreas or isolated islets have clearly demonstrated that GIP stimulates insulin secretion (15). Furthermore, administration of GIP in vivo has been revealed to increase insulin secretion in the presence of hyperglycemia (16). Therefore, GIP is identified as incretin and can stimulate insulin secretion by a different way from glucose (Fig. 2). However, the physiological significance of GIP had not been revealed until the GIP receptor–deficient mice were developed (17).

We have generated and characterized GIP receptor–deficient mice (17). Batch incubation studies using isolated pancreatic islets showed that GIP stimulated insulin secretion 2.9-fold from the islets of wild-type mice but had no insulinotropic effect in the GIP receptor–deficient mice, confirming the absence of GIP signaling in the mice.

After intraperitoneal glucose loading, blood glucose excursion was not significantly different between wild-type and the GIP receptor–deficient mice. On the contrary, after oral glucose loading, the peak levels of blood glucose were delayed and significantly higher, and insulin levels at 15 min after glucose challenge were significantly lower in GIP receptor–deficient mice (Fig. 3). The in vitro perfusion of pancreas and static incubation of islets confirmed that insulin secretions stimulated by glucose in the wild-type and the GIP receptor–deficient mice were comparable (17,18). Therefore, the difference of insulin excursion between wild-type and GIP receptor–deficient mice reflects the insulin secretion induced by GIP. Thus, insulin secretion from the pancreatic β-cells is regulated not only by glucose but also by GIP, a physiological factor with incretin action, especially in the postprandial phase.

GLUCAGON-LIKE PEPTIDE-1 AND GIP: ADDITIVE AS INCRETIN

Several experiments, including immunoneutralization of GIP (19), indicated the presence of another incretin in addition to GIP, and glucagon-like peptide 1 (GLP-1), a product of the glucagon gene that is expressed in the L-cells of lower small intestine, has been shown to be insulinotropic (20–23). Scrocchi et al. (24) produced GLP-1 receptor–deficient mice and found that the GLP-1 receptor–deficient mice have mild glucose intolerance accompanied with impaired insulin secretion, especially in the early phase of glucose loading, indicating that GLP-1 also has a physiological role in the regulation of postprandial insulin secretion.

Both GIP receptor–deficient and GLP-1 receptor–deficient mice exhibited only mild glucose intolerance after an oral glucose challenge. Therefore, we developed double incretin receptor–deficient mice with complete loss of both GIP and GLP-1 signaling (25). An oral glucose tolerance test revealed that blood glucose levels of double incretin receptor–deficient mice were higher, compared with that of wild-type mice or mice lacking a single incretin receptor and that the plasma insulin levels were lower, indicating that GLP-1 and GIP additively stimulate insulin secretion after meal ingestion as physiological insulinotropic factors.

Because GLP-1 and GIP are inactivated shortly after their secretion by DPP-IV, the inhibitors of DPP-IV could activate incretin signaling by enhancing endogenous levels of GIP and GLP-1 and decrease the blood glucose levels. The DPP-IV inhibitors significantly reduced glycemic excursion after oral glucose loading, not only in wild-type mice, but also in single incretin receptor–deficient mice. In contrast, the DPP-IV inhibitors had no effect on blood

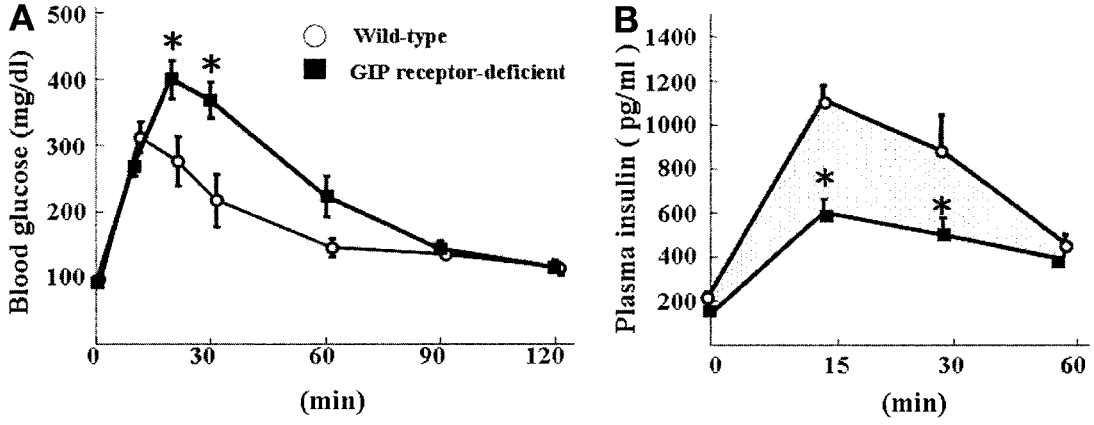

FIG. 3. Oral glucose tolerance test of GIP receptor–deficient mice. GIP receptor–deficient and wild-type mice in the C57BL/6 background were orally administered by 2 g/body wt kg glucose, and blood glucose (*A*) and plasma insulin (*B*) levels were determined at the indicated time. Dotted area after glucose loading represents GIP-induced insulin secretion. *$P < 0.05$. Adapted from Miyawaki et al. (17).

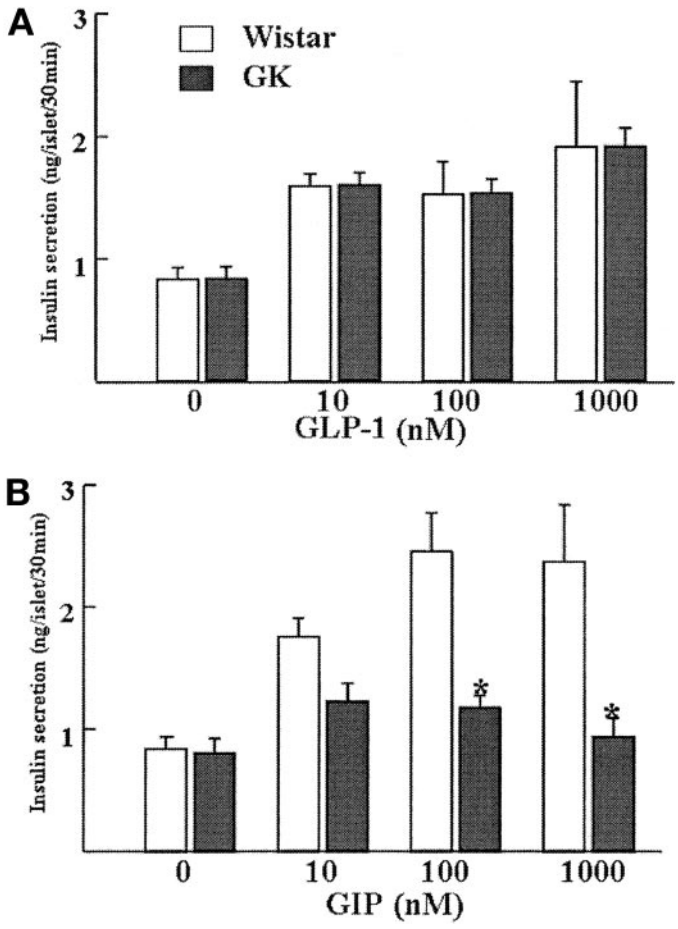

FIG. 4. GIP and GLP-1 effects on insulin secretion from islets of GK rats. Pancreatic islets were isolated from diabetic GK or control Wistar rats (8–10 weeks old) under pentobarbital anesthesia, and insulin release from intact islets was monitored using batch incubation as previously described (28). Islets were incubated with the indicated concentrations of GLP-1(7-36) amide (*A*) and GIP (*B*) in the presence of 8.3 mmol/l glucose for 30 min. *$P < 0.05$.

glucose and plasma insulin levels in double incretin receptor–deficient mice. These results indicated that it is essential and sufficient for DPP-IV inhibitors to activate either GLP-1 or GIP signaling, to achieve the acute glucose-lowering effects (25).

IMPAIRED INSULINOTROPIC EFFECTS OF GIP ON DIABETES

Type 2 diabetic patients exhibit an impaired incretin effect (26). Of particular note is that the effectiveness of GLP-1 intravenously administered to type 2 diabetic patients is preserved, whereas that of GIP is markedly reduced (27).

We have compared the activities of GLP-1 and GIP in GK rats, a nonobese model of type 2 diabetes. RT-PCR measurements of GLP-1 and GIP receptor mRNA revealed that receptor expression was not changed in GK rats. However, insulin release from GK rat islets was similar to what was observed in diabetic patients, with preservation of the response to GLP-1 and reduction of the GIP effects (Fig. 4).

Because gene expression of GIP receptor is unchanged, mutation of the GIP receptor gene may be involved in the selective impairment of GIP signaling in type 2 diabetic patients. We have investigated the entire coding region of the GIP receptor gene by PCR–single-strand conformation polymorphism (29) and identified one missense mutation, G198C, in exon 7. Function of the mutant GIP receptor was examined in Chinese hamster ovary (CHO) cells expressing the GIP receptor with G198C, revealing that cAMP response induced by different concentrations of GIP was right shifted, compared with wild-type GIP receptor–expressing CHO cells. However, the allelic frequencies of G198C were not significantly different: 1.9 and 2.0% in type 2 diabetic patients and control subjects, respectively. Further studies would be necessary to understand the molecular mechanisms of selective impairment of GIP signaling in type 2 diabetic patients.

EXTRAPANCREATIC EFFECTS OF INCRETIN

Incretin is defined as the hormonal factor(s) transmitting signals from the gut to pancreatic β-cells, and it had generally been thought that the principal role of GIP is to stimulate insulin secretion from pancreatic β-cells. Although the GIP receptor is expressed in other tissues than pancreatic β-cells, including adipose tissues (30) and osteoblasts (31), GIP actions on extrapancreatic tissues had received little attention. The comprehensive analyses of GIP receptor–deficient mice revealed the significance of the extrapancreatic effects of GIP (32–34).

GIP ACTION ON ADIPOCYTES

Adipose tissues play a crucial role not only in storing excess energy as triglyceride but also in secreting a variety of bioactive substances, such as leptin and adiponectin, and affecting glucose and fat metabolism. The expression of the GIP receptor on adipocytes was demonstrated in 1998 (30), and plasma GIP concentrations have been shown to be elevated in obese type 2 diabetic patients (35) and obese diabetic *ob*/*ob* mice (36). Furthermore, in vitro studies revealed that GIP stimulates the synthesis and secretion of lipoprotein lipase in rat adipose tissue, which hydrolyzes lipoprotein-associated triglycerides to produce free fatty acids available for local uptake (37). Using the GIP receptor–deficient mice, we revealed that GIP is an obesity-promoting factor, directly linking overnutrition to obesity (32).

High-fat diet is one of the environmental determinants of obesity. The wild-type and the GIP receptor–deficient mice were then fed a control diet or a high-fat diet. On a control diet, body weights of the wild-type and the GIP receptor–deficient mice remained similar; on a high-fat diet, the wild-type mice exhibited 35% body weight gain in the 50-week period and showed marked visceral and subcutaneous fat mass and liver steatosis. In contrast, neither weight gain nor such adiposity was observed in high fat–fed GIP receptor–deficient mice (Fig. 5). In conjunction with the insulin tolerance test, we concluded that inhibition of the GIP signal prevents obesity as well as insulin resistance. Because high fat–fed GIP receptor–deficient mice showed similar energy intake, energy expenditure was evaluated by measuring the respiratory quotient and oxygen consumption. After 3 weeks of high-fat feeding, the GIP receptor–deficient mice exhibited a significant reduction of respiratory quotient during the light phase, indicating that fat is used as preferred energy substrate in the GIP receptor–deficient mice and is not efficiently accumulated in adipocytes. After another 3 weeks on the high-fat diet, the wild-type mice consumed less oxygen than the GIP receptor–deficient mice during the light phase. These results clearly show that the resistance to obesity of the GIP receptor–deficient mice was

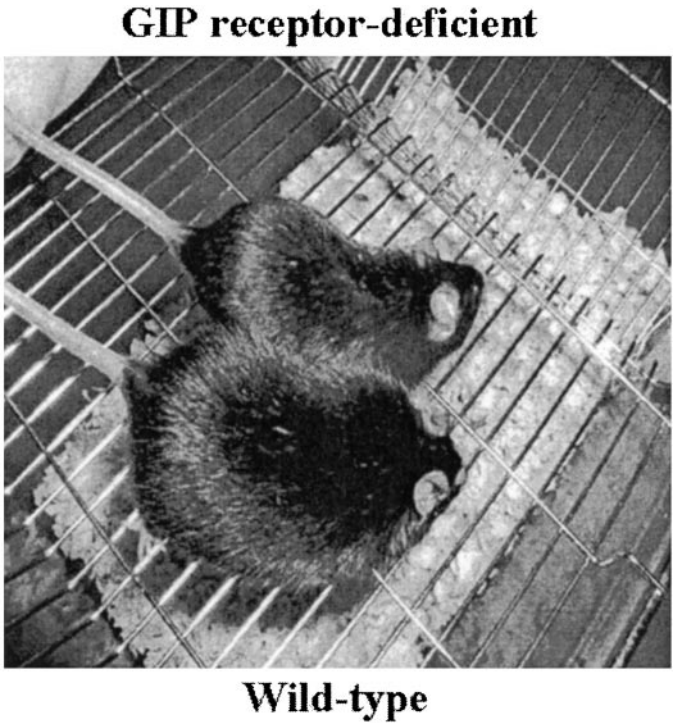

FIG. 5. Gross appearance of wild-type and GIP receptor–deficient mice on a high-fat diet. Wild-type (*lower*) and GIP receptor–deficient (*upper*) mice were fed a high-fat diet for 50 weeks.

due to higher energy expenditure rather than lower energy intake.

Hyperphagia is another environmental determinant of obesity. Because obese *ob/ob* mice have much elevated adiposity due to hyperphagia caused by mutation of the leptin gene and exhibited diabetes with marked insulin resistance and dyslipidemia, we crossbred the GIP receptor–deficient mice with *ob/ob* mice and generated GIP receptor–deficient *ob/ob* mice (32). Genetic ablation of GIP signaling ameliorates not only obesity through increasing energy expenditure, but also insulin insensitivity, glucose intolerance, and dyslipidemia without decreasing insulin secretion (Fig. 6), indicating again the importance of GIP signaling in the adiposity and glucose and lipid homeostasis. These results are consistent with the recent finding that chemical ablation of GIP signaling using GIP receptor antagonist against *ob/ob* mice improves glucose tolerance and ameliorates insulin resistance (38).

Therefore, GIP has a physiological role on nutrient uptake into adipocytes and is a key molecule linking overnutrition to obesity. Excessive intake of fat induces hypersecretion of GIP, which increases nutrient uptake in the adipocytes and causes obesity and insulin resistance. In the absence of GIP signaling, fat is not efficiently accumulated in adipocytes and instead is used predominantly as the preferred energy source.

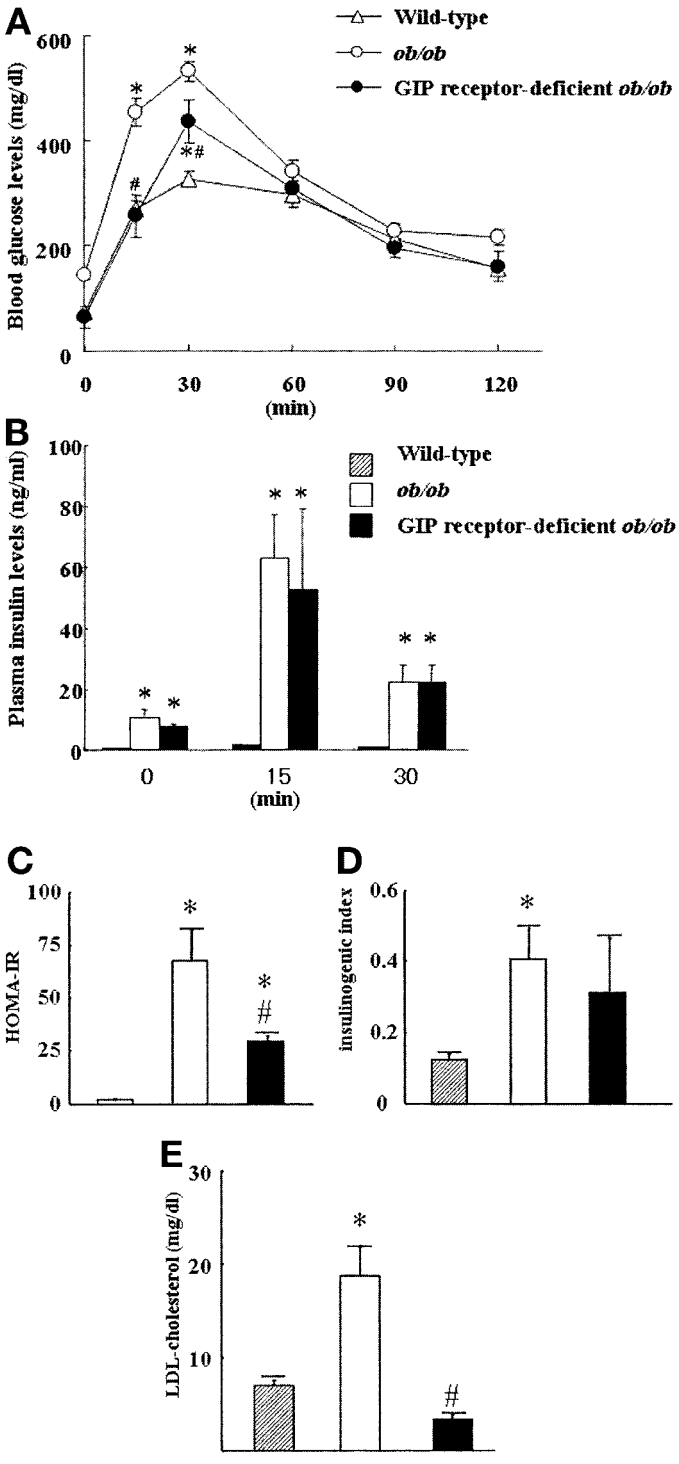

FIG. 6. GIP receptor–deficient *ob/ob* mice. Blood glucose (*A*) and plasma insulin (*B*) excursions after oral glucose loading, insulin resistance evaluated by homeostasis model assessment–insulin resistance (HOMA-IR) (*C*), insulin secretion evaluated by insulinogenic index (*D*), and LDL cholesterol levels (*E*) were compared among wild-type, *ob/ob*, and GIP receptor–deficient *ob/ob* mice. **P* < 0.05 vs. wild-type mice; #*P* < 0.05 vs. *ob/ob* mice.

GIP ACTION ON OSTEOBLASTS

Bone plays a crucial role in the body's nutrient reserve of calcium to maintain blood calcium levels in addition to its structural role. Old bone is continuously resorbed by the hematopoietically derived osteoclasts, and new bone is formed from the mesenchyme-derived osteoblasts, which is called bone remodeling (39). Because the GIP receptor is expressed in osteoblasts (31), we have examined the effects of GIP on bone using GIP receptor–deficient mice (34). Growth of GIP receptor–deficient mice is similar to that of wild-type mice in both male and female, and there is no significant difference in soft X-ray images of the skeleton. However, histological analysis revealed that the bone trabeculae of GIP receptor–deficient mice are thin-

ner than those of wild-type mice, which is compatible to the histological feature of osteoporosis. Bone histomorphometrical analyses revealed that bone formation parameters were significantly lower and that the number of osteoclasts was significantly increased, indicating that GIP receptor–deficient mice have high-turnover osteoporosis. In vitro examination showed the percentage of osteoblastic cells undergoing apoptosis to be significantly decreased in the presence of GIP. These data suggest that the gut hormone GIP stimulates bone formation by protecting osteoblasts from apoptosis directly through the GIP receptor. Furthermore, GIP receptor–deficient mice exhibited an increased plasma calcium concentration after meal ingestion. Although adequate intake of calcium is essential, calcium supplementation alone has only a partial effect in preventing bone loss (40–42) and little is known about the molecular mechanisms of the pathway from meal ingestion to calcium deposition in bone. Our study indicates that the metabolically thrifty GIP gene promotes not only efficient storage of ingested fat in adipose tissues but also of ingested calcium in bone.

GIP ACRONYM AND PHYSIOLOGY

GIP was originally isolated for its ability to influence gastric acid secretion and was designated as gastric inhibitory polypeptide (43). Presently, the same acronym GIP is given the alternate designation of glucose-dependent insulinotropic polypeptide because of its ability to stimulate insulin secretion (15,16). Comprehensive analysis of GIP receptor–deficient mice revealed that an insulinotropic effect is only one of the physiological roles of GIP and that GIP also has physiological roles on fat accumulation into adipose tissues and calcium accumulation into bone. All of these effects constitute physiology of GIP, and we propose the acronym GIP for gut-derived nutrient-intake polypeptide.

CLINICAL APPLICATION OF GIP FOR TREATMENT OF DIABETES

Type 2 diabetes is characterized by various degrees of insulin resistance and pancreatic β-cell dysfunction. In Europe and the U.S., insulin resistance with obesity is a predominant pathological condition of diabetes, whereas impaired insulin secretion is predominant in Asia. GIP receptor agonists in addition to DPP-IV inhibitors could have a good indication against diabetes with impaired insulin secretion, especially in Asia, and GIP receptor antagonists could have a good indication against diabetes with insulin resistance, especially in Europe and the U.S.

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