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# Novel extrapancreatic effects of incretin

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## Keywords

Gastric inhibitory polypeptide, Glucagon-like peptide-1, Incretin

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## INTRODUCTION

Incretins are hormones that stimulate insulin secretion from pancreatic  $\beta$ -cells, which are responsive to the difference of insulin levels after oral and intravenous ingestion of glucose. Gastric inhibitory polypeptide (GIP) was originally isolated as a 42-amino-acid hormone from the duodenum, inhibiting gastric acid secretion and gastric motility<sup>1,2</sup>. Subsequently, GIP was shown to be released from intestinal K cells and to stimulate insulin secretion from pancreatic  $\beta$ -cells directly, and was renamed glucose-dependent insulinotropic polypeptide<sup>3,4</sup>. Glucagon-like peptide-1 (GLP-1) was originally identified as a glucagon-like polypeptide from the glucagon precursor<sup>5</sup>. GLP-1 (7-37) and GLP-1(7-36) amide were shown to be released from intestinal L cells, and stimulate insulin secretion from pancreatic  $\beta$ -cells directly<sup>6</sup>. Amino acid sequences of human GIP and GLP-1<sup>7,8</sup> were shown to be highly homologous, belonging to the glucagon/secretin family.

## GLP-1 AND GIP HAVE PANCREATIC EFFECTS

Receptors for GLP-1 and GIP were cloned and shown to be coupled with the stimulatory G protein, belonging to the glucagon/secretin receptor family<sup>9,10</sup>. Genetic deficiency of GLP-1 receptor (GLP1R) and GIP receptor (GIPR) in mice was developed<sup>11–13</sup>. As shown in Figure 1, GLP1R-deficient mice and GIPR-deficient mice showed higher blood glucose levels with impaired initial insulin response after oral glucose loading.

## ABSTRACT

The hormonal factors implicated as transmitters of signals from the gut to pancreatic  $\beta$ -cells are referred to as incretins. Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretins. In addition to the insulinotropic effects, we have shown, using the GIP receptor and GLP-1 receptor-deficient mice, that GIP and GLP-1 have direct actions on adipocytes and the kidney, respectively. Because GIP receptors and GLP-1 receptors are differentially expressed in a tissue-specific manner, GIP and GLP-1 have specific physiological activities, and further comprehensive characterization of the extrapancreatic actions of GIP and GLP-1 is anticipated, as dipeptidyl peptidase IV inhibitors activate both GIP and GLP-1 signaling.

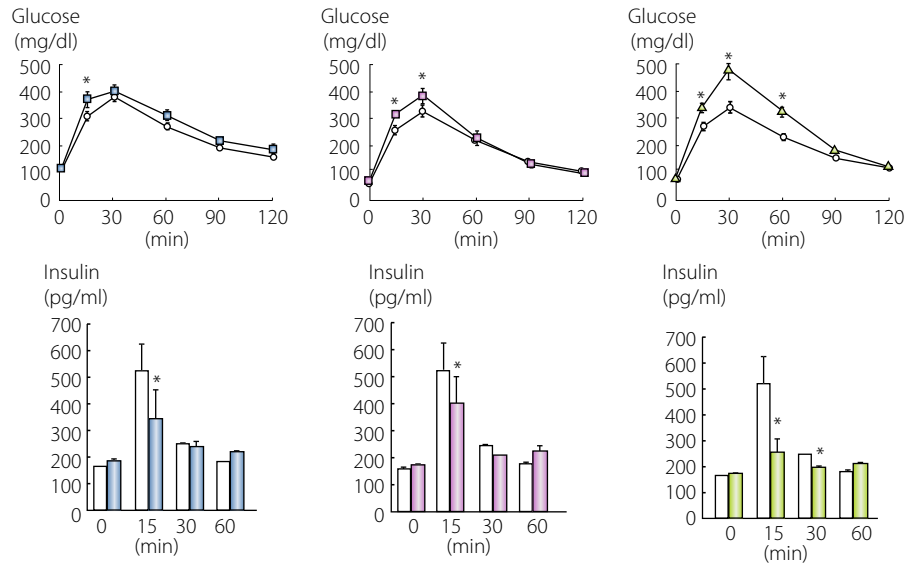
These results confirm that GLP-1 and GIP act as incretin. Simultaneous ablation of GLP1R and GIPR further increased the blood glucose levels and decreased initial insulin response after glucose loading, showing that GLP-1 and GIP cannot compensate adequately for each other.

## GLP-1 AND GIP HAVE EXTRA-PANCREATIC EFFECTS

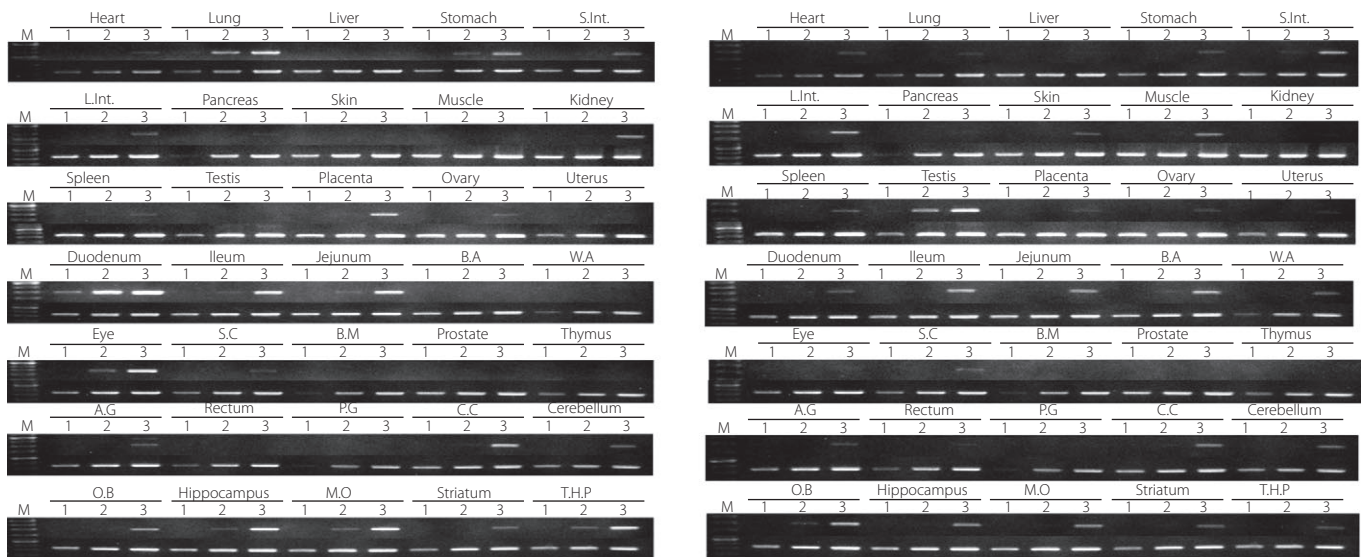
Receptors for GLP-1 and GIP are expressed not only in pancreatic  $\beta$ -cells, but also in several extrapancreatic tissues. Figure 2 shows the tissue-specific expression patterns of GLP-1 and GIP receptors in mice. GLP1R is highly expressed in the lung and duodenum of mice, and GIPR is highly expressed in the testis of mice. The expression patterns of GLP1R and GIPR are quite different, suggesting that GLP-1 and GIP have their own physiological activities.

The second amino acid from the NH<sub>3</sub>-terminal of GLP-1 and GIP is the alanine residue, and *in vitro* and *in vivo* studies showed that both GLP-1 and GIP are substrates of dipeptidyl peptidase IV (DPP-4), and that active peptides are degraded to inactive peptides<sup>14,15</sup>. Mice separately deficient in GLP1R or GIPR could augment insulin secretion after treatment with DPP-4 inhibitors, one class of antihyperglycemic agents widely used for treatment of diabetes, but the mice simultaneously deficient in GLP1R and GIPR could not respond to the treatment, showing that both GLP-1 and GIP signaling can be augmented by treatment with DPP-4 inhibitors. These results suggest that the extrapancreatic effects of GLP-1 and GIP might be stimulated by treatment with DPP-4 inhibitors, in addition to the increased pancreatic effects. Furthermore, DPP-4-resistant GLP1R agonists, another class of antihyperglycemic agents,

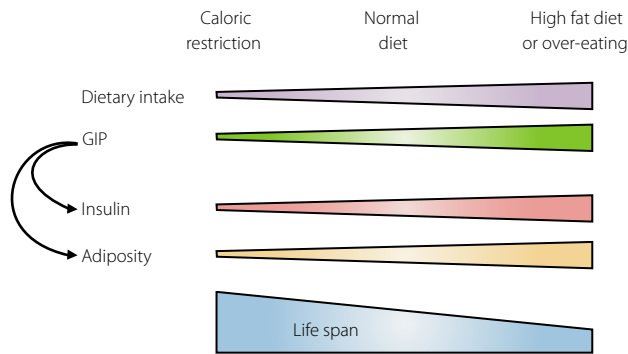
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**Figure 1** | Incretin deficiency and glucose intolerance. Glucagon-like peptide-1 (GLP-1) receptor-deficient (left, blue), gastric inhibitory polypeptide (GIP) receptor-deficient (middle, red) and double receptor-deficient mice (right, green) in the C57BL/7 background were challenged with oral glucose, and levels of glucose and insulin levels were measured. \* $P = 0.05$  versus wild-type mice. Original mice are described in references<sup>11–13</sup>.



**Figure 2** | Expression patterns of incretin receptor. Gene expression of (a) glucagon-like peptide-1 receptor and (b) gastric inhibitory polypeptide receptor was examined using reverse transcription polymerase chain reaction. Complementary deoxyribonucleic acid templates from various mouse tissues (Genostaff, Tokyo, Japan) were amplified 25 cycles (lane 1), 30 cycles (lane 2) and 35 cycles, and fractionated on 1.5% agarose gels with polymerase chain reaction products of glyceraldehyde-3-phosphate dehydrogenase as controls. Heart, lung, liver, stomach, small intestine (S.Int.), large intestine (L.Int.), pancreas, skin, skeletal muscle, kidney, spleen, testis, placenta, ovary, uterus, duodenum, ileum, jejunum, brown adipose (BA), white adipose (WA), eye, spinal cord (S.C), bone marrow (B.M), prostate, thymus, adrenal gland (A.G), rectum, pituitary gland (P.G), cerebral cortex (C.C), cerebellum, olfactory bulb (O.B), hippocampus, medulla oblongate (M.O), striatum and thalamus + hypothalamus + pons (T.H.P) were examined.



**Figure 3** | Gastric inhibitory polypeptide as a gut-derived satiation responsive polypeptide.

stimulate the pancreatic and extrapancreatic effects of GLP-1. Therefore, comprehensively understanding the pancreatic and extrapancreatic effects of GLP-1 and GIP is essential.

### GIP AS GUT-DERIVED SATIATION-RESPONSIVE POLYPEPTIDE

As aforementioned, GIP was shown to have an activity to inhibit gastric acid secretion and was known as gastric inhibitory polypeptide. Subsequent studies showed that GIP has an activity to stimulate insulin secretion in a glucose-dependent manner, thus it was renamed glucose-dependent insulinotropic polypeptide. However, glucose-dependent insulinotropic polypeptide might be an imprecise name, in two regards. First, GIP has several extrapancreatic effects in addition to stimulation of insulin secretion. Second, several peptides, including GLP-1, can stimulate insulin secretion in a glucose-dependent manner.

Our group has taken particular note of the extrapancreatic effects of GIP. As GIPR is expressed in white adipose tissues, and GIP increases glucose uptake and heparin-releasable lipoprotein lipase activity of the differentiated 3T3-L1 adipose cell line<sup>16</sup>, we hypothesized that diet-induced GIP is responsive for promoting nutrient uptake into the adipose tissues. A high-fat diet or overeating induces obesity in mice as well as humans, compared with the control diet (Figure 3). These diet styles increase blood GIP levels and insulin levels<sup>16</sup>. We bred GIP receptor-deficient mice fed a high-fat diet or induced by leptin deficiency to overeat, and found that mice lacking the GIPR were protected from obesity. The GIPR-deficient mice had a lower respiratory quotient, suggesting that fat was used as the preferred energy substrate instead of storing it in the adipose tissues. Therefore, GIP directly links overnutrition to obesity. Next, we examined the effects of aging on obesity, as aging is associated with increased fat mass and decreased lean mass. Aged GIPR-deficient mice on the normal diet showed significantly reduced fat mass with conserved lean mass. Furthermore, GIPR-deficient mice showed higher heart rate, lower body temperature and increased physical activity<sup>17</sup>. These phenotypic characterizations of genetic inactivation of GIP signaling

showed similarity with those of caloric-restricted mice. Indeed, the GIP-deficient mice showed lower adiposity<sup>18</sup>. From these results and other studies, we have proposed GIP as “gut-derived satiation-responsive polypeptide.”

### GLP-1 AS A RENOPROTECTIVE FACTOR

Diabetic patients might develop microvascular complications, such as retinopathy, nephropathy, and neuropathy; and macrovascular complications, such as cerebral infarction, myocardial infarction and peripheral arterial diseases, which are the major causes of morbidity and mortality. Because poor glucose control is the major risk factor for diabetic complications, antihyperglycemic agents, such as sulfonylureas, biguanides and insulin, have been given to diabetic patients.

Although GLP-1 receptor messenger ribonucleic acid was detected in the kidney, its precise localization was controversial because of the poor specificity of anti-GLP1R antibodies<sup>19</sup>. Using *in situ* hybridization, we have shown that GLP-1 receptor messenger ribonucleic acid was expressed in glomerular capillary and vascular walls in the mouse kidney<sup>20</sup>. *In vivo* examination was carried out using Akita diabetic mice on KK/Ta or C57BL/6 background. Akita diabetic mice on C57BL/6 background are nephropathy-resistant, and genetic ablation of GLP1R showed higher urinary albumin levels and more advanced mesangial expansion with increased glomerular superoxide and upregulated renal nicotinamide adenine dinucleotide phosphate oxidase, despite comparable levels of hyperglycemia. By contrast, nephropathy-prone Akita diabetic mice on KK/Ta background treated with liraglutide, a GLP1R agonist, showed reduced albuminuria and mesangial expansion. These results showed that GLP-1 has a crucial role in protection against increased renal oxidative stress under chronic hyperglycemia. Although it has not been defined as the primary end-point of the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus–Thrombolysis in Myocardial Infarction 53 trial<sup>21</sup>, patients treated with saxagliptin, a DPP-4 inhibitor, were significantly more likely to have an improved albumin-to-creatinine ratio, consistent with our animal studies.

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### DISCLOSURE

The authors declare no conflict of interest.

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