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Kyoto University
Glucose-dependent insulinotropic polypeptide deficiency reduced fat accumulation and insulin resistance, but deteriorated bone loss in ovariectomized mice

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Keywords
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INTRODUCTION
Glucose-dependent insulinotropic polypeptide (GIP) is a gut hormone released from enteroendocrine K cells that enhances insulin secretion after food intake1. The GIP receptor is expressed in pancreatic β-cells, and other tissues including adipose tissue and bone2–4. We previously generated GIP-deficient mice, and found that GIP deficiency protected the mice from high-fat diet-induced obesity and insulin resistance5, suggesting that blocking GIP signaling might be a strategy to treat obesity. However, mice lacking GIP showed signs of osteopenia, characterized by reduced bone volume, reduced number of trabeculae and increased osteoclast numbers5. Ovariectomy accelerates osteopenia and fat accumulation in the abdominal region6,7, and leads to metabolic abnormalities, such as insulin resistance and dyslipidemia8,9; however, the mechanisms remain unclear.

To further investigate the role of GIP in fat, glucose and bone metabolism, we evaluated the effect of GIP deficiency on adipose tissue and bone metabolism in the setting of ovariectomy in mice.

METHODS
Animal care and procedures were approved by Kyoto University Animal Care Committee (MedKyo16584).

GIP gene expression was reduced in C57BL/6J GIPgfp/+ mice or was entirely absent in GIPgfp/gfp mice compared with wild-type (WT) mice, which were all housed as described previously5. Surgical ovariectomies (dorsal approach) were carried out to female WT, GIPgfp/+ and GIPgfp/gfp mice at the age of 8 weeks. Experiments were carried out on three separate cohorts of mice, each consisting of three groups of five to seven mice. Body fat mass, food intake along with energy expenditure and locomotor activity were measured as described previously10,11. Oral glucose tolerance tests (OGTTs) were carried out to 17 and 37 weeks-of-age using 2 g/kg body weight glucose, and insulin tolerance tests were carried out to 24 and 40 weeks-of-age using 0.5 U/kg regular insulin as described previously10. Plasma insulin, total GIP and glucagon-like polypeptide-1 (GLP-1) levels were measured using a mouse
insulin enzyme-linked immunosorbent assay kit (Shibayagi, Gunma, Japan), GIP enzyme-linked immunosorbent assay kit (EMD Millipore Corporation, Billerica, MA, USA) and total GLP-1 enzyme-linked immunosorbent assay kit (Meso Scale Discovery, Rockville, MD, USA), respectively. Bone analysis by dual-energy X-ray absorptiometry and microcomputed tomography (µCT; LCT-100M, Aloka, Tokyo, Japan), and the measurement of plasma osteocalcin and C-terminal telopeptide of type I collagen using a mouse osteocalcin EIA kit (Biomedical Technologies Inc., Stoughton, MA, USA) and RatLapsTM EIA kit (Immunodiagnostic Systems Inc, Gaithersburg, MD, USA) were carried out at 16 weeks-of-age. The blood samples were collected from the tail vein without anesthesia.

All data are expressed as the mean ± standard error of the mean. Statistical analysis was carried out using one-way ANOVA with the Tukey–Kramer multiple comparison test. P-values <0.05 were considered significant.

RESULTS

Body weight gain after ovariectomies was tracked in cohort 2 (Figure 1a). The body weight of GIPgfp/gfp mice was significantly lower than WT mice from 25 weeks-of-age, but there was no difference between WT and GIPgfp/+ mice throughout the study. As expected, the uterus showed atrophy in all ovariectomized mice (data not shown). Both subcutaneous and visceral fat depots were reduced by ~40% in GIPgfp/gfp mice, but not significantly reduced in GIPgfp/+ mice compared with those in WT mice at 26 weeks-of-age in cohort 1 (Figure 1b). Lean body weight, food intake, locomotor activity and energy expenditure were not different among all three groups (Figure 1b–e).

Blood glucose levels during OGTTs at 17 weeks-of-age in cohort 1 were not different (Figure 2a). Insulin levels were decreased at 30 min after glucose administration in GIPgfp/gfp mice compared with WT mice, but the area under the curves (AUC) of plasma insulin responses were not different (Figure 2b). The AUC of plasma GIP were under the detection level in GIPgfp/gfp mice, and the AUC of GIP responses were similar in WT and GIPgfp/+ (Figure 2c,g). By 37 weeks-of-age in cohort 2, blood glucose levels were significantly decreased in GIPgfp/gfp mice compared with WT, resulting in a lower AUC (Figure 2e). In contrast, insulin responses to oral glucose were not different among the three groups (Figure 2f). Plasma GLP-1 levels during OGTT were not significantly different in WT and GIPgfp/gfp mice (15.81 ± 2.55 and 11.95 ± 6.26 pg/dL at 15 min after OGTT, respectively). There were no differences in glucose reduction in response to exogenous insulin administration among the three groups at either 24 or 40 weeks-of-age (Figures 2d,h). The ovariectomized WT mice showed GIP hypersecretion, obesity and insulin resistance compared with non-ovariectomized WT mice (Figure S1).

At 16 weeks-of-age in cohort 3, body length and bone mineral density measured by dual-energy X-ray absorptiometry, whole and cortical bone mineral density as determined by microcomputed tomography, and plasma C-terminal telopeptide of type I collagen levels were not different between the three groups (Table 1). However, cancellous bone mineral density, cortical thickness and plasma osteocalcin levels were decreased by 64%, 50% and 38% in GIPgfp/gfp mice compared with WT mice, respectively. Cortical thickness and plasma osteocalcin levels were decreased by 43% and 27% in GIPgfp/gfp mice compared with GIPgfp/+ mice, respectively, whereas there was no difference in GIPgfp/+ mice compared with WT mice.

DISCUSSION

Ovarian hormone deficiency increases abdominal fat, insulin resistance and osteopenia.8,9,12 We investigated the role of GIP in a rodent ovariectomy model, and the combined effect of ovarian hormone deficiency and GIP deficiency. We found that weight gain, subcutaneous and visceral fat mass, cancellous bone mineral density, bone cortical thickness, and plasma osteocalcin levels were reduced in GIP knockout mice compared with WT mice. These results are consistent with previous findings in GIP receptor knockout mice, GIP receptor antagonists, chemical K-cell ablation, and GIP antibody therapy showing the anabolic effect of GIP on adipose tissue5,13–15 and bone.16

Although we did not detect significant changes in food intake or locomotor activity, we cannot exclude the possibility that small reductions in food intake or increase in locomotor activity contributed to reduced weight gain in GIPgfp/gfp mice. We also observed improved glucose intolerance in GIPgfp/gfp mice aged 37 weeks-of-age compared with WT mice with the same magnitude of insulin responses, whereas no difference was seen at 17 weeks-of-age. Perhaps the significantly lower body weight and lower visceral fat mass in GIPgfp/gfp mice compared with WT mice after 26 weeks-of-age might have contributed to these results. We have previously reported that GLP-1 secretion remained unchanged in GIPgfp/gfp mice, and the present study also showed no compensatory hypersecretion of GLP-1 in the ovariectomized GIP-deficient mice.

We did not detect any improvement in insulin sensitivity, which might have been expected with reduced fat accumulation, but we cannot exclude the possibility of subtle changes in insulin sensitivity that could not be detected by our whole-body insulin tolerance tests. We speculate that glucose homeostasis is not dramatically impaired in the GIP-deficient mice, because lower body weight and visceral fat mass improved insulin sensitivity.

The present study did not show any significant changes in glucose and bone metabolism in GIPgfp/+ mice compared with WT mice. These results were different from previous findings on partial reduction of GIP signaling.5,17 Although we used GIPgfp/+ mice in which GIP levels were reduced before ovariectomies (Figure S2), the levels of GIP at OGTT were similar to that of WT mice after ovariectomies. The mechanisms of how ovariectomy might influence GIP production in GIPgfp/+ mice are unknown, but potentially, changes in estrogen or gonadotropin hormones might alter GIP secretion.
Regarding the role of GIP on bone metabolism, reduced bone formation, decreased bone strength and bone quality have been reported in GIP receptor knockout mice18–21, and conversely, GIP-overexpressing transgenic mice showed increased bone mass22. Although there is a report of osteocalcin-induced release of glucagon-like peptide-123, no report that GIP regulates osteocalcin directly exists as far as we know. Ovarian hormone deficiency induced osteopenia itself, but GIP deficiency suppressed bone formation. We have to consider not only estrogen deficiency, but also elevated gonadotropins might contribute to bone metabolism. There are very few reports of the relationship between GIP and estrogen deficiency. In humans, plasma GIP levels were approximately twice as high in postmenopausal women as young premenopausal women24, and estrogen replacement therapy reduced plasma GIP levels in postmenopausal women25. We could investigate only a part of the relationship between GIP and estrogen in the present study.
**SHOR T REPORT**

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**Figure (a)**: AUC-Blood glucose

![Graph showing AUC-Blood glucose with Time (min) on the x-axis and Blood glucose (mg/dL) on the y-axis.](image)

**Figure (b)**: AUC-Insulin

![Graph showing AUC-Insulin with Time (min) on the x-axis and Insulin (ng/ml) on the y-axis.](image)

**Figure (c)**: AUC-GIP

![Graph showing AUC-GIP with Time (min) on the x-axis and Total GIP (pg/mL) on the y-axis.](image)

**Figure (d)**: Glucose levels (%)

![Graph showing Glucose levels (%) with Time (min) on the x-axis.](image)

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**Figure (e)**: AUC-Blood glucose

![Graph showing AUC-Blood glucose with Time (min) on the x-axis and Blood glucose (mg/dL) on the y-axis.](image)

**Figure (f)**: AUC-Insulin

![Graph showing AUC-Insulin with Time (min) on the x-axis and Insulin (ng/ml) on the y-axis.](image)

**Figure (g)**: AUC-GIP

![Graph showing AUC-GIP with Time (min) on the x-axis and Total GIP (pg/mL) on the y-axis.](image)

**Figure (h)**: Glucose levels (%)

![Graph showing Glucose levels (%) with Time (min) on the x-axis.](image)
Table 1 | Bone analysis in ovariectomized mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>GIP&lt;sup&gt;gfp/+&lt;/sup&gt;</th>
<th>GIP&lt;sup&gt;gfp/gfp&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length (cm)</td>
<td>9.4 ± 0.02</td>
<td>9.4 ± 0.06</td>
<td>9.4 ± 0.07</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>25.5 ± 0.82</td>
<td>25.8 ± 1.02</td>
<td>23.5 ± 0.72</td>
</tr>
<tr>
<td>BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22.2 ± 1.1</td>
<td>23.6 ± 1.5</td>
<td>20.7 ± 1.3</td>
</tr>
<tr>
<td>Whole BMD (mg/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>3603 ± 28.2</td>
<td>3255 ± 8.0</td>
<td>3084 ± 19.6</td>
</tr>
<tr>
<td>Cortical BMD (mg/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>3589 ± 22.7</td>
<td>3317 ± 93</td>
<td>3282 ± 6.2</td>
</tr>
<tr>
<td>Cancellous BMD (mg/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2878 ± 63.7</td>
<td>1483 ± 143</td>
<td>1044 ± 22.4*</td>
</tr>
<tr>
<td>Cortical thickness (cm)</td>
<td>0.074 ± 0.008</td>
<td>0.066 ± 0.009</td>
<td>0.038 ± 0.001***</td>
</tr>
<tr>
<td>Plasma osteocalcin (ng/mL)</td>
<td>47.8 ± 3.1</td>
<td>40.1 ± 3.2</td>
<td>29.4 ± 2.1*, ***</td>
</tr>
<tr>
<td>Plasma CTx (ng/mL)</td>
<td>16.9 ± 0.71</td>
<td>17.0 ± 0.53</td>
<td>17.9 ± 0.51</td>
</tr>
</tbody>
</table>

Data presented as the mean ± standard error of the mean. BMD, bone mineral density; CTx, C-terminal telopeptide of type I collagen. *P < 0.05 versus wild-type mice (WT). **P < 0.01 versus WT. ***P < 0.05 versus green fluorescent protein (GFP) inserted into the glucose-dependent insulinotropic polypeptide (GIP) locus, in which the GIP locus was reduced (GIP<sup>gfp/+</sup>) and absent (GIP<sup>gfp/gfp</sup>) mice.

but the mechanism by which estrogen modulates GIP production requires further study.

In conclusion, the present study supports the concept that the total elimination of GIP might reduce weight gain and improve glucose metabolism, but could be associated with undesirable consequences on bone loss in the setting of ovariec-
tomy in mice.

ACKNOWLEDGMENTS

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DISCLOSURE

NI served as a medical advisor for Takeda, Taisho, GlaxoSmithKline and Mitsubishi Tanabe; lectured for MSD, Sanofi, Novartis, Dainippon Sumitomo, Kyowa Kirin and Mitsubishi Tanabe; and received payment for services, outside the submitted work. The other authors declare no conflict of interest.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | OGTT and ITT in ovariectomized WT (WT) mice and non-ovariectomized WT (WT sham) mice (n = 4–6). Body weight of WT mice and WT sham mice were 44.2 ± 2.1 g and 28.2 ± 1.4 g, respectively (P < 0.01). *P < 0.05, **P < 0.01 compared to WT sham. GIP, glucose-dependent insulinotropic polypeptide; HOMA-IR, homeostasis model assessment of insulin resistance; WT, wild-type. Data are expressed as means ± standard error of the mean.

Figure S2 | OGTT in female 9 weeks of age in WT, GIP<sup>+/+</sup> and GIP<sup>+/−</sup> mice (n = 7). *P < 0.05, **P < 0.01 compared to WT. GIP, glucose-dependent insulinotropic polypeptide; GFP, green fluorescent protein; WT, wild-type. Data are expressed as means ± standard error of the mean.