<center>学位論文要約</center>

論文題目　The effects of nutrition intake on intestinal mucosal repair
　　　　　and metabolic regulation through gut hormones
　　　　　（栄養摂取の消化管ホルモンを介した腸管粘膜修復ならびに代謝調節に及ぼす影響）

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　　　　Various hormones secreted from different enteroendocrine cells in the gastrointestinal tract regulate nutrient absorption and metabolism, and the intestinal environment. Among them, gastric inhibitory polypeptide (GIP) which is secreted from K-cells in the proximal small intestine and glucagon-like peptide 1 (GLP-1), secreted from L-cells in the distal small intestine and colon, are recognized as incretins which amplify insulin secretion from pancreatic beta-cells in a glucose-dependent manner. Incretins are responsible for about half of the total postprandial insulin secretion. GLP-2 （glucagon-like peptide 2） is co-secreted along with GLP-1 from L-cells. GLP-2 does not have insulinotropic effect as GLP-1. On the other hand, it has been shown that GLP-2 is an intestinotrophic hormone.

　　　　It is well known that not only carbohydrates, but also fat and protein ingestion stimulate incretin secretion. A recent study of our research group demonstrated that GIP level is increased dose-dependently in response to oral glucose load, and that GIP secretion after mixed meal ingestion (meal containing carbohydrates, fat and proteins) is higher than that after oral glucose load. GLP-1 secretory response is, however, not significantly different between oral glucose load and mixed meal load. These findings suggest that GIP secretion is more susceptible to nutritional composition than GLP-1 secretion. In addition, amino acids have been shown to stimulate incretin secretion. We also reported that GIP secretion was positively associated with body mass index and early-phase insulin secretion, and GLP-1 secretion was negatively associated with plasma glucose levels. Thus, incretin secretion is affected by ingestion of various kinds of nutrients and their combinations, which in turn might affect differently GIP and GLP-1 secretion.

　　　　A dietary supplementation product enriched with glutamine, dietary fiber, and oligosaccharide (GFO) is widely applied for enteral nutrition support in

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Japan. We already estimated GIP, GLP-1, and GLP-2 levels after GFO ingestion in human study, and found that GFO stimulates GLP-2 as well as GLP-1 secretion (unpublished data). In addition, human GLP-2 reduces the severity of colonic injury in a murine model of dextran sulfate sodium (DSS)-induced colitis. Therefore, we speculate that GFO stimulates GLP-2 secretion concomitantly with GLP-1 secretion and attenuates the development of mucosal damage of ulcerative colitis (UC) via an enhancement of GLP-2 secretion.

As previously described, the postprandial plasma GIP level is greatly augmented in response to an intake of a meal containing abundant fat rather than simply glucose. We also reported that GIP plays a critical role in maintaining the blood glucose levels by inducing hypersecretion of insulin in high-fat diet (HFD)-induced obesity. In addition, GIP receptor (GIPR) is expressed in adipose tissue and increases glucose and triglyceride uptake in fat cells. Thus, GIP has both direct and indirect effects on the accumulation of energy into adipose tissue. Deficiency of GIPR signaling ameliorates high-fat diet-induced obesity due to a lack of direct and indirect GIP effects. However, it is unknown whether direct GIP action on adipose tissue contributes to adiposity and body weight gain under HFD-feeding i*n vivo*.

Thus, the aim of the present study is to confirm the inhibitory effect of GFO on UC by using mice with experimental colitis (Study 1) and to clarify the effects of GIP on adipose tissue by using adipose tissue specific GIP receptor-deficient mice (Study 2).

Study 1
Background and aims: The supplementation product GFO enriched with glutamine, dietary fibers, and oligosaccharide, is widely used for enteral nutrition support. Given the intestinotrophic effects, GFO is also expected to attenuate the symptoms of UC. In this study, we investigated whether GFO has suppressive effects on mucosal damage in UC in a mouse experimental model. We also investigated the effect of GFO on glucagon-like peptide secretion.
Materials and methods: C57BL/6 mice received 2.5% of DSS in drinking water for 5 days to induce colitis. They then were given 0.25 mL of GFO or 20% of glucose solution twice daily for 10 days. Another set of mice receiving unaltered drinking water was used as normal control. In addition, we evaluated the effect of single oral administration of GFO on plasma GLP-1 levels in mice.
Results: Body weight loss and disease activity index were significantly lower in

GFO-treated mice compared to those in glucose-treated mice ($p < 0.05$). DSS-induced reduction of colon length was significantly alleviated in GFO-treated mice compared to that in glucose-treated mice ($p < 0.01$). In addition, histological findings revealed that intestinal inflammation was significantly attenuated in mice treated with GFO. Furthermore, treatment with GFO significantly inhibited DSS-induced increase in mRNA expression of interleukin-1 beta. In a single oral administration, plasma GLP-1 levels were significantly higher in GFO-administered mice compared to those in glucose-administered mice.

Conclusion: These results suggest that GFO is effective in preventing the progression of mucosal damage in UC. We speculate that GFO stimulates GLP-2 secretion concomitantly with GLP-1 secretion and attenuates the development of mucosal damage of UC via enhancement of GLP-2 secretion.

Study 2

Background and aims: GIP is an incretin. GIP has a direct effect on the adipose tissue where it induces energy accumulation. Inhibition of GIPR signaling prevents the development of obesity and insulin resistance induced by HFD. However, it remains unclear whether direct GIP action in the adipose tissue contributes to adiposity in vivo. In this study, we generated adipose tissue-specific GIPR-deficient mice (GIPR$^{adipo-/-}$) and clarified the direct GIP action in this tissue.

Materials and methods: GIPR$^{adipo-/-}$ mice were generated from floxed GIPR mice (GIPR$^{fl/fl}$) and aP2-Cre transgenic mice. GIPR$^{adipo-/-}$, GIPR$^{fl/fl}$, and aP2-Cre mice were fed HFD for 15 weeks. Afterwards, oral glucose tolerance test (OGTT), insulin tolerance test (ITT), CT (computer tomography) scan, and immunohistochemistry (in liver and adipose tissue) were performed. Also, triglyceride (TG) content in liver was measured.

Results: Expression levels of GIPR mRNA were particularly lower in visceral and subcutaneous adipose tissues of GIPR$^{adipo-/-}$. Body weight gain under HFD was significantly lower in GIPR$^{adipo-/-}$ compared to that of control mice (GIPR$^{fl/fl}$ and aP2-Cre). Fat mass and adipocyte size did not differ between GIPR$^{adipo-/-}$ and control mice. OGTT data showed that glucose and insulin levels were low in GIPR$^{adipo-/-}$, although there was no significant difference. HOMA-IR (homeostasis model assessment of insulin resistance) and ITT data showed that insulin sensitivity was improved in GIPR$^{adipo-/-}$ compared to control mice. Furthermore,

fat content in liver was significantly lower in GIPR$^{adipo-/-}$ compared to control mice.

Conclusion: The direct effect of GIP on the adipose tissue plays an important role in HFD-induced insulin resistance *in vivo.*

In the present study, we investigated the intestinotrophic and metabolic effects of gastrointestinal hormones. Firstly, we focused on the effect on the GLP-2 secreted concomitantly with GLP-1, and secondly, we focused on the GIP. GIP links overnutrition with obesity. Our experiment first showed that the direct effect of GIP on the adipose tissue plays an important role in HFD-induced insulin resistance. On the other hand, GLP-1 receptor agonists used for the treatment of type 2 diabetes are associated with reductions of HbA1c, fasting plasma glucose, and body weight. Therefore, we can expect that certain types of food, such as GFO, which stimulate GLP-1 secretion and reduce GIP secretion, are effective in treatment of metabolic diseases. Thus, regulation of gastrointestinal hormones, such as GIP, GLP-1, and GLP-2, using various nutrients components can be a very important and promising therapeutic approach for intestinal and metabolic disorders.