博士論文の要約

Adaptive changes in postprandial glucagon-like peptide-1 response and its role during the progression of diet-induced obesity and diabetic state in rats

（食事誘導性肥満および糖尿病モデルラットにおける食後 glucagon-like peptide-1 分泌応答の変動とその役割）

Jukkrapong Pinyo

Laboratory of Nutritional Biochemistry,

Graduate School of Agriculture, Hokkaido University

General introduction

Excess energy intake, especially from high fat and/or high sucrose diet, promotes the development of overweight and obesity. Nowadays, obesity has become a major public health problem, and the incidence of the disease is increasing rapidly in the world population. In addition, obesity is associated with metabolic syndrome disorders, including hyperglycemia, insulin resistance, and type 2 diabetes mellitus. Not only metabolic syndrome disorders, obesity has been previously reported to alter gut hormone secretion.

Glucagon-like peptide-1 (GLP-1) is a gut peptide hormone. GLP-1, a product of the proglucagon gene, is primarily secreted in two forms, such as GLP1-(7-37) NH_2 and GLP-1-(7-36) NH_2 from enteroendocrince L-cells in the lower small intestine and the colon in response to nutrients in the ingested diets including glucose, fatty acids, proteins,

amino acids, or dietary fibers. GLP-1 is well recognized as an “incretin hormone” that regulates postprandial glucose homeostasis through stimulating insulin secretion. Gut hormones have various physiological functions under normal conditions, however, changes and roles of gut hormones in the development of obesity or diabetic condition have not been clearly identified. In addition, direction of postprandial GLP-1 secretion (enhanced, unchanged, or decreased) is still under debate in response to nutrients ingestion in obese, pre-diabetic, and diabetic patients, including in animal models. Understanding the relationship between GLP-1 secretory responses and metabolic status during the progression of diet-induced obesity (DIO) may provide valuable information applicable to the management or prevention of glucose intolerance, obesity, and type 2 diabetes in the future. It is difficult to conduct DIO in human study in a designed experiment. Therefore, animal experiments are advantageous to explore the mechanisms involved in the development of metabolic diseases including obesity and diabetes. In order to determine adaptive changes in postprandial GLP-1 during the progression of DIO and diabetic state, animal models continuously fed obesogenic diets were employed in the present studies in this thesis. Accordingly, it was possible to monitor the changes in postprandial response such as glucose, insulin, GLP-1 levels, and gastric emptying during obesity development.

The purposes of overall study were to clarify (1) the alteration of postprandial GLP-1 secretion and (2) role of postprandial GLP-1 secretion during progression of diet-induced obesity, and (3) mechanism underlying the adaptive changes in GLP-1 responses.

Chapter 1

The aims of experiment 1 was to examine GLP-1 response to meal ingestion

during the DIO progression and to determine whether a high fat or a high sucrose diet largely attribute to obesity (adiposity) and/or glucose intolerance development. Each rat was housed in an individual cage under temperature and humidity controlled environment with a 12 h light–dark cycle (8.00 a.m. to 8.00 p.m. light period) and was allowed free access to diet and water (*ad libitum*) except the day before experiment. The rats were divided into four groups based on body weight (BW), plasma glucose, and GLP-1 concentrations and were given a control, a high fat and high sucrose (HFS, 30% fat and 40% sucrose) diet, a high fat (HiFat, 30% fat) diet, and a high sucrose (HiSuc, 40% sucrose) diet, respectively, totally for 5 weeks. The final compositions of 30% fat in a HiFat diet and 40% sucrose in a HiSuc diet were adjusted to be equal to those in a HFS diet (30% fat and 40% sucrose). BW and food intake (weighing uneaten food) were measured every 2 days.

Until now, postprandial glycemic and insulin responses are generally tested using the classical models such as oral glucose tolerance test (OGTT) and MTT. However, MTT is more appropriate methodology for investigating postprandial metabolic responses, compared to OGTT. In order to mimic the dietary exposure in normal life, MTT was used to assess the postprandial response in this study. In addition, voluntary ingestion is more relevant than enforced gavage feeding. Therefore, the rats were given a control diet at the dose of 10 g/kg BW during MTT experiment. In this study, MTT was conducted at 2 and 4 weeks after feeding the test diet in order to examine postprandial glucose, insulin, and GLP-1 responses. After 5 weeks of feeding period, blood samples from portal vein and abdominal aorta were taken under sodium pentobarbital anesthesia. The rats were then sacrificed and tissue samples were collected.

The results showed that body weight gain of HiFat group and adipose tissue weight

of HFS group were increased, compared to that in the control group. These results suggest that high fat diet had a potent impact on obesity (adiposity) development rather than high sucrose diet. Nevertheless, a combination of high fat and high sucrose diet had an intense impact on obesity development than that of individual high fat diet consumption alone. In both MTTs, postprandial glycemic response showed a similar level in all groups. After 2 weeks of feeding the test diet, HFS and HiFat diet feeding groups showed a significant elevation of the postprandial GLP-1 level at 15 min, compared to basal level after control diet ingestion, while other groups had only small (insignificant) increment in GLP-1, suggesting that the sensitivity of L-cells in the small intestine to luminal nutrients (protein, carbohydrates, fatty acids) was enhanced by the chronic feeding of HFS and HiFat diet. In addition, chronic feeding of HFS and HiFat diet for 2 weeks much highly affected to postprandial insulin response whereas HiSuc feeding group showed no any impact on these parameters Likewise, the area under the curve (AUC) of homeostasis model assessment of insulin resistance (HOMA-IR) was largely increased by chronic HFS diet feeding for 2 weeks. It indicates that chronic HFS diet feeding immediately triggered insulin resistance development. Interestingly, continuous consumption of HiSuc diet until 4 weeks showed that postprandial GLP-1, insulin, and HOMA-IR were similar to those observed in HiFat diet feeding group. Chronic feeding of HiSuc diet gradually increased postprandial GLP-1 and insulin secretion including HOMA-IR index suggesting that HiSuc feeding requires the time to develop metabolic impairment. From overall experiment 1, the results suggest that excessive ingestion of an HFS diet rapidly caused adaptive changes in nutrient sensitivity in GLP-1-producing cells rather than an individual HiFat or HiSuc diet alone. However, a HiFat diet likely has a relatively potent effect on GLP-1 response compared with a HiSuc diet, which may play a role in the normalizing

postprandial glycemia and retarding the development of glucose intolerance.

Chapter 2

Due to the incretin effect (stimulating insulin secretion), increased GLP-1 concentrations could contribute to normalize the postprandial glucose homeostasis. In addition, incretin-based therapy is effectively used for type 2 diabetes treatment. Therefore, increasing GLP-1 secretion may be a promising target for prevention and treatment of glucose intolerance. In addition, the results of experiment 1 showed that GLP-1 secretion in response to normal diet administration was increased by chronic consumption of obesogenic diet during the progression of DIO.

Hence, the aim of experiment 2 was to clarify the role of enhanced endogenous GLP-1 secretion in glucose intolerance and obesity development. The male SD rats (5-week-old) were given the control or the HFS diet with or without continuous administration of exendin-9 (Ex9, 100 μg/day), a GLP-1 receptor antagonist, for 5 weeks. Ex9 is a specific and competitive antagonist of the GLP-1 receptor, which has similar structure to GLP-1 and is widely used to block the GLP-1 signaling at its receptor in various studies. In this study, the GLP-1 receptor antagonist (Ex9) or vehicle (0.09% NaCl) was chronically delivered to the rats throughout the experimental period (35 days) by using an osmotic pump implanted subcutaneously. In experiment 1, some of the rats did not consume the provided diet (10 g/kg BW, that is enough to consume within 30 min after overnight or 16 hour fasting according to preliminary experiment) during MTT. It might be due to acute stress response after basal blood collection. Hence, MTTs by an oral administration of a liquid diet (15 kcal/10 mL/kg BW) were performed instead of re-feeding of a powder diet (Experiment 1).

The results demonstrated that postprandial GLP-1 levels in the HFS and Ex9 groups were slightly higher after 2 weeks of feeding period and significantly higher after 4 weeks of feeding period than those of the control group. The results confirmed that chronic consumption of an HFS diet enhances postprandial GLP-1 secretion in response to the diet administration. Although both the HFS and Ex9 groups showed elevated postprandial GLP-1 secretion compared to that in the control group, postprandial insulin secretion was diminished in the Ex9 group compared to the HFS group. These results clearly demonstrate that postprandial GLP-1 was effectively blocked by Ex9 treatment, resulting in insufficient insulin secretion. Accordingly, the results illustrate that chronic HFS feeding with continuous Ex9 administration rapidly established postprandial hyperglycemia, while HFS alone maintained a glycemic response similar to the control group in both the 2 and 4 weeks of feeding periods. These results suggest that the enhancement of the postprandial GLP-1 secretion play an important role in maintaining the normal postprandial glycemia during the DIO development.

Chapter 3

On the other hand, it has been unclear whether the increased GLP-1 responses in the diet-induced obese models were sustained or disappeared during a prolonged diet-induced obesity. Also, the meal-induced GLP-1 response in genetically diabetic models under treatment with the obesogenic diet was unknown.

So, the aims of experiment 3 were to examine changes in adaptive enhancement of postprandial GLP-1 responses in the diet-induced obese rats during a long-term experiment (26 weeks) and to explore potential mechanisms involved in the adaptive changes in postprandial GLP-1 responses in DIO and glucose intolerance status both in

non-diabetic and diabetic rat models. Goto-Kakizaki (GK) rats are recognized as non-obese diabetic rodent model and widely used to investigate the development of type 2 diabetes and its complications. It was developed by selective breeding of glucose intolerant Wistar rats over many generations, are spontaneously polygenic model. GK rats are considered as one of the best type 2 diabetic animal models because they present many similarities with type 2 diabetic patients such as hyperglycemia, impaired glucose-induced insulin secretion (insulin resistance), decreased β-cell mass, and decreased insulin sensitivity.

In this study, the non-diabetic Wistar and diabetic GK rats (5-week-old) were fed either a control diet or a HFS diet for 26 weeks. MTT was performed for monitoring postprandial glucose, insulin, and GLP-1 responses after a liquid diet administration (15 kcal/kg BW) every 4 or 8 weeks after feeding the obesogenic diet. After 26 weeks of test diet feeding, the rats were sacrificed. The intestinal mucosa and tissue were then collected in order to measure mRNA expression and GLP-1 content.

The results demonstrated that postprandial glycemic responses of Wistar rats fed an HFS diet (WH) were maintained similar to Wistar rats fed the control diet (WC) in the early period of DIO and were then significantly increased after 16 weeks of feeding period. The postprandial GLP-1 and insulin responses in the WH group were higher or tended to be higher than those in the WC group throughout the study. Although GK rats fed with an HFS diet (GH) had higher glycemic responses, compared to GK rats fed with the control diet (GC), GH and GC groups had similar postprandial GLP-1 and insulin responses throughout the study. These results demonstrate that the enhanced postprandial GLP-1 response was sustained but not impaired after prolonged treatment with an obesogenic diet. Furthermore, adaptive changes of the postprandial GLP-1 response did not occur in

the genetic diabetic model rats. The difference in the GLP-1 responses between Wistar and GK rats could be involved in the development of glucose intolerance. Moreover, the jejunal and ileal GLP-1 contents were increased in response to chronic HFS feeding only in Wistar rats, but not in GK rats, which may responsible for the increased postprandial GLP-1 secretion. Based on these results, it is likely that L-cells in the jejunum and ileum are the major sources of postprandial GLP-1 secretion. In experiment 1, the results showed that HFS and HiFat diet had a potent effect on postprandial GLP-1 response rather than HiSuc diet feeding. Hence, the mRNA expression of free fatty acid receptors (*Ffar1*, *Ffar2*, *Ffar3*, and *Ffar4*) was then investigated in the present study. *Ffar1* mRNA expression level in the jejunum of WH rats tended to be higher than that of WC rats. The results suggest that chronic HFS diet feeding enhanced the sensitivity to long chain fatty acids possibly in GLP-1 producing cells. Although specific reasons are not entirely clear, *Ffar4* mRNA was almost undetectable in GK rats regardless of diet consumption. In contrast to intestinal *Ffar4* mRNA expression, pancreatic *Ffar4* mRNA expression showed a similar level in both diabetic and non-diabetic rats. Thus, the development of glucose intolerance in GK rats might be partly due to *Ffar4* deficiency in the intestine. From overall experiment 3, the results suggest that the postprandial GLP-1 responses were enhanced and sustained, but not attenuated in the DIO in normal rats after a long experimental period. Moreover, failure of adaptive enhancement of GLP-1 response in GK rats could be responsible for exacerbating glucose impairment.

Conclusion

In overall conclusions, a HiFat diet rather than a HiSuc diet has a potent impact on postprandial GLP-1 response and obesity (adiposity) development in rats. Moreover,

HFS diet showed much impact on these parameters compared to the HiFat diet alone. The enhancement of GLP-1 secretion during obesity development plays an important role in maintaining normal postprandial glycemia through increasing insulin secretion. However, the adaptation was absent in the diabetic GK rats, implying that failure of adaptive enhancement of GLP-1 response accelerates the development of glucose intolerance. Increased GLP-1 production and fatty acid sensitivity in the small intestine are potent factors that are involved in enhanced GLP-1 responses under obesity.

Although the underlying mechanisms involved in the increment of postprandial GLP-1 secretion are still not clearly elucidated, possible involvement of increased fatty acid receptor expression and/or GLP-1 production in the small intestine were suggested. Moreover, upstream pathways and other possible pathways need to be further clarified. However, understanding the relationship between GLP-1 secretory responses and metabolic status described in these studies could contribute to a better design of therapeutic and preventive approaches for obesity and type 2 diabetes. Enhancing GLP-1 secretion may hold the key for management of obesity and type 2 diabetes. Manipulating the diet, supplementary ingredients, and/or therapeutics in a way to promote the interactions with free fatty acid receptors could increase GLP-1 secretion and enhance its beneficial effects for obesity and diabetes. Finally, the potential side effects of any treatments should be further considered before applying to humans.