Nutrition 62 (2019) 122–130

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Continuous feeding of a combined high-fat and high-sucrose diet, rather than an individual high-fat or high-sucrose diet, rapidly enhances the glucagon-like peptide-1 secretory response to meal ingestion in diet-induced obese rats

Jukkrapong Pinyo M.Sc. [a], Tohru Hira Ph.D. [a,b,*], Hiroshi Hara Ph.D. [a,b]

[a] Graduate School of Agriculture, Hokkaido University, Sapporo, Japan
[b] Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

ARTICLE INFO

Article History:
Received 30 July 2018
Received in revised form 12 December 2018
Accepted 8 January 2019

Keywords:
Glucagon-like peptide-1
Luminal nutrients
L-cell
Meal tolerance test
Obesity

ABSTRACT

Objectives: Glucagon-like peptide-1 (GLP-1) is secreted by enteroendocrine L-cells in response to nutrient ingestion. To date, GLP-1 secretion in diet-induced obesity is not well characterized. We aimed to examine GLP-1 secretion in response to meal ingestion during the progression of diet-induced obesity and determine whether a combined high-fat and high-sucrose (HFS) diet, an individual high-fat (HiFat), or a high-sucrose (HiSuc) diet affect adaptive changes in the postprandial GLP-1 response.

Methods: Rats were fed a control, HiFat diet (30% weight), HiSuc diet (40% weight), or HFS (30% fat and 40% sucrose) diet for 5 wk. Meal tolerance tests were conducted to determine postprandial glucose, insulin, and GLP-1 responses to standard (control) diet ingestion every 2 wk.

Results: After 5 wk, body weight gain of the HiFat (232.3 ± 7.8 g; $P = 0.021$) and HFS groups (228.0 ± 7.8; $P = 0.039$), but not the HiSuc group (220.3 ± 7.9; $P = 0.244$), were significantly higher than that of the control group (200.7 ± 5.4 g). In meal tolerance tests after 2 wk, GLP-1 concentration was significantly elevated in the HFS group only (17.2 ± 2.6 pM; $P < 0.001$) in response to meal ingestions, but the HiFat group (16.6 ± 3.7 pM; $P = 0.156$) had a similar response as the HFS group. After 4 wk, GLP-1 concentrations were similarly elevated at 15 min in the HFS (14.1 ± 4.4; $P = 0.010$), HiFat (13.2 ± 2.0; $P < 0.001$), and HiSuc (13.0 ± 3.3; $P = 0.016$) groups, but the HFS (9.8 ± 1.0; $P = 0.019$) and HiFat (8.3 ± 1.5; $P = 0.010$) groups also had significant elevation at 30 min.

Conclusions: These results demonstrate that the continuous ingestion of excessive fat and sucrose rapidly enhances the GLP-1 secretory response to luminal nutrients, and the HiFat diet may have a potent effect compared with the HiSuc diet on GLP-1 secretory responses. The increment of postprandial GLP-1 and insulin secretion may have a role in normalizing postprandial glycaemia and slowing the establishment of glucose intolerance.

Introduction

Obesity is rapidly increasing in the global population and has become a major public health problem [1]. Dietary habits are now considered key factors related to obesity development, especially excessive energy consumption from high-fat (HiFat) diets and/or high-sucrose (HiSuc) diets [2,3]. Obesity leads to metabolic syndromes, such as hyperinsulinemia, hypertension, hyperlipidaemia, and type II diabetes mellitus [4,5]. Glucagon-like peptide-1 (GLP-1), a product of the proglucagon gene, is a gut-derived hormone that is produced and secreted from enteroendocrine L-cells in response to nutrient ingestion, including protein, glucose, fatty acids, and dietary fiber [5–9]. GLP-1 has been recognized as an incretin hormone that reduces the postprandial glycaemic response by stimulating insulin secretion [10]. Existing data on GLP-1 responses to nutrient ingestion (enhanced, unchanged, and decreased) in patients with obesity, prediabetes, and diabetes remain unclear [11,12]. GLP-1 secretion in response to oral glucose was enhanced in patients with type II diabetes [13]. In contrast, GLP-1 secretion was impaired in obese subjects and patients with

Sources of support: This work was supported by JSPS KAKENHI (Grant Number 16K07725).
Conflicts of interest: The authors declare no conflicts of interest.
* Corresponding author. Tel.: +81 11 706 2811; fax: +81 11 706 2811.
E-mail address: Hira@chem.agr.hokudai.ac.jp (T. Hira).

https://doi.org/10.1016/j.nut.2019.01.004
0899-9007/

type II diabetes [14,15], but GLP-1 secretion in response to oral glucose was unchanged in patients with type II diabetes [16].

In order to understand how GLP-1 responses are modified in the process of diet-induced obesity, the adoption of animal models that are continuously fed an obesogenic diet, rather than using genetically obese or diabetic models, is relevant. Continuous feeding (8 wk) of combined high-fat and high-sucrose diets (HFS) increased postprandial GLP-1 secretion in response to normal meal administration, as previously reported [17]. Although the impact of feeding animal models with a HiFat and/or HiSuc diet has been previously examined, the alteration of the postprandial GLP-1 response during obesity development has not been clearly characterized [18–20].

The aims of this study were to clarify GLP-1 response to meal ingestion during the progression of diet-induced obesity, and determine whether a combined high-fat and high-sucrose (HFS) diet or an individual HiFat or HiSuc diet contributed to adaptive changes in postprandial GLP-1 secretion. In this study, rats were given a standard (control) diet as a meal tolerance test (MTT) instead of the typical oral glucose tolerance test (OGTT).

Experimental methods

Animals and diet

Five-wk-old male Sprague-Dawley rats (weighing 160-200 g) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and fed with the American Institute of Nutrition–93 G (control) diet for 1 wk as an acclimation period [21]. Each rat was housed in an individual cage in a temperature- and humidity-controlled environment with a 12 h light–dark cycle (0800-2000 h light period) and allowed free access to diet and water. After a 1-wk acclimation period, fasting glucose and GLP-1 concentrations were measured. Then, the rats were divided into four groups with matched body weight, plasma glucose, and GLP-1 concentrations.

Rats in each group were given the control, HFS (30% fat weight and 40% sucrose weight) [17], HiFat (30% fat), or HiSuc (40% sucrose) diet (Table 1) for 5 wk. The initial body weight, fasting glucose, and GLP-1 concentrations were 168.7 ± 3.1 g, 73.2 ± 2.4 mg/dL, and 18.0 ± 3.0 pM in the control group (n = 10); 169.3 ± 2.5 g, 72.0 ± 3.3 mg/dL, and 17.7 ± 3.1 pM in the HFS group (n = 8); 169.1 ± 4.3 g, 71.5 ± 4.3 mg/dL, and 17.8 ± 1.8 pM in the HiFat group (n = 10); and 167.7 ± 2.8 g, 71.5 ± 2.0 mg/dL, and 20.0 ± 3.2 pM in the HiSuc group (n = 8), respectively.

Table 1
Test diet composition

Ingredient	Control	HFS	HiFat	HiSuc
		g/kg of diet		
Cornstarch	397.486	–	167.486	97.486
Casein*	200	200	200	200
Dextrinized cornstarch†	132	–	132	132
Sucrose	100	399.486	100	400
Soybean oil	70	70	70	70
Lard oil	–	230	230	–
Fiber (cellulose)‡	50	50	50	50
Mineral mixture§	35	35	35	35
Vitamin mixture§	10	10	10	10
L-Cystine	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5
tert-Butylhydroquinone	0.014	0.014	0.014	0.014
Protein (energy %)	20.5	15.9	15.9	20.5
Carbohydrate (energy %)	63.6	31.3	31.3	63.6
Fat (energy %)	15.9	52.8	52.8	15.9
Energy density (kcal/g)	3.96	5.11	5.11	3.96

HFS, high-fat and high-sucrose diet; HiFat, high-fat diet; HiSuc, high-sucrose diet.
*Acid Casein (Fonterra, Ltd., Auckland, New Zealand).
†TK-16 (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan).
‡Avicel PH102 (Asahi Kasei Chemicals Corporation, Tokyo, Japan).
§Mineral and vitamin mixtures were prepared in accordance with the American Institute of Nutrition–93 G formulation.

The final compositions of 30% fat in the HiFat diet and 40% sucrose in the HiSuc diet were adjusted to be equal to those in the HFS diet. Body weight and food intake were measured every 2 d. All experimental animal procedures used in the study were approved by the institutional animal care committee, and the animals were maintained in accordance with the institutional animal guidelines for the care and use of laboratory animals.

Meal tolerance test

The MTT was performed on rats after they were fed the test diets for 2 and 4 wk to determine the postprandial glucose, insulin, and GLP-1 responses to standard meal ingestion. The rats fasted for 16 h, and then basal (0 min) blood samples were collected from the tail vein. Then, the rats were given the control diet (10 g/kg body weight) for 30 min, and blood samples from the tail vein were collected at 15, 30, 60, 90, and 120 min after consumption of the diet. In this study, only rats that ingested >90% of the given diet were used to assess the postprandial responses.

Blood samples were collected in chilled tubes that contained heparin (final concentration: 50 IU/mL; Ajinomoto Company, Inc., Tokyo, Japan) and aprotinin (final concentration: 500 Kallikrein inhibitor units [KIU]/mL; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma was separated by centrifugation at 2300 × *g* for 10 min at 4°C and stored at -80°C until the time of the analysis. The plasma glucose level was determined with the Glucose CII Test Kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin and GLP-1 concentrations were analysed using the rat insulin enzyme-linked immunosorbent assay (ELISA; AKRIN-010T; Shibayagi Company Limited, Gunma, Japan) and multi-species GLP-1 total ELISA (EZGLP1 T-36K; Merck Millipore, Darmstadt, Germany), respectively. The GLP-1 total ELISA detects both GLP-1 (7-36) and GLP-1 (9-36) and has no significant cross-reactivity with GLP-2, GIP, glucagon, or oxyntomodulin. The minimum detection limit of the assay is 1.5 pM. The intra-assay precision is <5%, and the interassay precision <12%.

The homeostatic model assessment of insulin resistance (HOMA-IR) was used to assess insulin resistance and calculated using the following equation [22] with glucose and insulin values at the fasting state and values of the area under the curve (AUC) during MTT:

$$HOMA-IR = \text{insulin}(\mu U/mL) \times \text{glucose}(mg/dL)/2430$$

where 1 mg insulin = 26 IU.

Blood and tissue collection

After receiving the test diet for 5 wk, the rats fasted overnight (16 h). Blood samples from the portal vein were taken under sodium pentobarbital anaesthesia (50 mg/kg of body weight, somnopentyl injection; Kyoritsu Seiyaku Corporation, Tokyo, Japan) and collected in a chilled syringe that contained heparin (final concentration: 50 IU/mL), aprotinin (final concentration: 500 KIU/mL), and dipeptidyl peptidase IV inhibitors (final concentration: 50 μmol/L; DPP4-010; Merck Millipore, Darmstadt, Germany).

The rats were sacrificed by exsanguination, and intestinal segments were carefully dissected and washed with a cold saline solution (0.9% NaCl). Subsequently, 2-cm segments of the jejunum, ileum, and colon were taken from the middle region to measure GLP-1 content. The caecal tissues were washed with a cold saline solution and divided equally into two parts. Next, 2 cm from the

middle region was collected for GLP-1 measurement. All intestinal segments were rapidly frozen in liquid N_2, and stored at -80°C until measurement of the GLP-1 content. The mesenteric, retroperitoneal, and epididymal adipose tissues were individually weighed and expressed as the visceral adipose tissue weight. Plasma was stored as previously described to measure the glucose, insulin, total GLP-1, triacylglycerol, and total cholesterol levels. Plasma triacylglycerol and cholesterol concentrations were measured using the triacylglycerol and cholesterol E test kits (Wako Pure Chemical Industries, Osaka, Japan), respectively.

Measurement of the GLP-1 content in intestinal tissue

Intestinal segments (2 cm in length) were immersed in an ethanol-acid solution (absolute ethanol:water:12 M HCl = 74:25:1, 5 mL/g of intestinal tissue segments) [23] and cut into small pieces. The tissue samples were homogenized at 25,000 rpm (Ultra Turrax homogenizer T18, IKA, Staufen, Germany) for 2 min and placed at 4°C for 24 h. Subsequently, the homogenates were centrifuged at 2000 × *g* for 20 min.

The supernatant was collected to measure the total GLP-1 and protein contents using the Multi Species GLP-1 total ELISA (EZGLP1T-36K; Merck Millipore, Darmstadt, Germany) and Pierce BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL), respectively. The supernatant was diluted 10-fold for protein measurement and 500-fold (jejunum, ileum, and colon segments) and 625-fold (colon segment) for GLP-1 measurement with normal saline solution.

Statistical analysis

The primary endpoint of this study was nutrient-induced GLP-1 responses in rats fed various (obesogenic) diets. The secondary endpoint was to assess glycaemic and insulin responses, body weight, and adipose tissue weight changes. The results were expressed as mean ± SEM. The sample size was calculated based on the experimental design (two-way repeated measure analysis of variance [ANOVA]) in MTTs to examine postprandial GLP-1 responses as the primary outcome measure by using G*Power software (version 3.1.9.2) with effect size f = 0.4 and power = 0.8. Significant effects of time (TI), treatment (TR), and interactions of time and treatment (TI × TR) were assessed by two-way repeated measure ANOVA in the results of MTTs. One-way ANOVA and Tukey-Kramer's tests were used to assess the significant difference among the treatments for parametric data (assessed by goodness of fit test). A Kruskal–Wallis test and Dunn's multiple range test were used for MTT data that contained nonparametric data. A Dunn's with control test was used to determine the significant differences from baseline (0 min) value within the same group. *P* values ≤ 0.05 were considered statistically significant. The statistical analysis was performed using JMP Pro, version 13 software (SAS Institute, Inc., Cary, NC).

Results

Basal glucose, insulin, GLP-1 levels, and HOMA-IR after feeding with test diet for 2 and 4 wk

After consuming the test diet for 2 and 4 wk, the rats fasted overnight (16 h) before conducting the MTT. After 2 wk of feeding, basal glucose (84.1 ± 4.5 to 90.0 ± 3.6 mg/dL; *P* = 0.396), insulin (0.11 ± 0.01 to 0.20 ± 0.06 nM; *P* = 0.329), GLP-1 (12.1 ± 1.5 to 20.4 ± 2.6 pM; *P* = 0.070), and HOMA-IR (0.54 ± 0.07 to 1.11 ± 0.34; *P* = 0.287) were not significantly different among all treatment groups (Figs. 1A, B, C, and D). After 4 wk, the basal insulin level and HOMA-IR tended to be higher in the HFS group (0.26 ± 0.03; *P* = 0.218; and 1.45 ± 0.20; *P* = 0.200) compared with the control group (0.17 ± 0.02 nM; and 0.90 ± 0.10; Figs. 1F and H).

Postprandial glycaemic, insulin, and GLP-1 responses during MTT

In this study, MTT was used instead of the OGTT to determine postprandial responses to ingestion of a standard diet (control diet). MTT can be used to mimic dietary exposure in normal life, and voluntary ingestion is more relevant than enforced gavage feeding. Although the HiSuc group had relatively lower concentrations, postprandial glycemic responses were overall similar in all groups (Figs. 2A and E) after receiving the test diet for 2 wk. The AUC of insulin increased largely in the HFS group (1.15 ± 0.19 nM; *P* = 0.015) compared with the control group (0.46 ± 0.04 nM). The HiFat group (0.93 ± 0.35 nM; *P* = 1.000) also showed a similar trend, but less so than the HFS group (Fig. 2F).

Insulin levels at 60 min were significantly higher in the HFS group (0.62 ± 0.08 nM) than those in the HiSuc (0.23 ± 0.03 nM; *P* = 0.022) and control (0.24 ± 0.03 nM; *P* = 0.016) groups (Fig. 2B). The basal levels of GLP-1 of the control, HFS, HiFat, and HiSuc groups were 20.4 ± 2.6 pM, 12.1 ± 1.5 pM, 12.6 ± 2.3 pM, and 18.2 ± 3.8 pM, respectively. Owing to the varied basal GLP-1 levels in each treatment group (Fig. 1), changes in GLP-1 concentrations from the basal value (ΔGLP-1) were presented to illustrate postprandial GLP-1 response in this study. The GLP-1 level significantly increased after 15 min in the HFS group (17.2 ± 2.6 pM; $P < 0.001$) compared with the basal level. The HiFat group also showed a similar level (16.6 ± 3.7 pM; *P* = 0.156) without a significant difference, but the HiSuc group (6.6 ± 4.8 pM, *P* = 1.000) was more similar to the control group (7.3 ± 2.3 pM; *P* = 0.169; Fig. 2C).

The AUC of HOMA-IR was calculated using the glycaemic and insulin responses (0-120 min) during the MTT to estimate the degree of insulin resistance in the postprandial state. The results revealed that the HFS group (22.4 ± 1.0; *P* = 0.008) had the highest AUC of HOMA-IR, followed by the HiFat group (18.3 ± 7.3; *P* = 1.000), but the HiSuc group (8.2 ± 0.9; *P* = 1.000) showed a similar level to the control group (8.9 ± 1.1; Fig. 2H).

After receiving the test diet for 4 wk, AUC of postprandial glycaemia in the HFS (301.6 ± 6.1 mg/dL), HiFat (293.0 ± 5.9 mg/dL), and HiSuc (291.6 ± 7.4 mg/dL) groups were almost similar to those in the control group (291.3 ± 6.6 mg/dL; *P* = 0.579). The HFS group (1.53 ± 0.18 nM; *P* = 0.010) had a higher postprandial insulin AUC than the control group (0.77 ± 0.06 nM), but the HiFat group (1.17 ± 0.14 nM; *P* = 0.676) showed an intermediate level between the HFS and control groups (Fig. 3B).

The GLP-1 response and HOMA-IR AUC in the HiSuc group showed similar values to those of the HiFat group in contrast with the result of the MTT after 2 wk (Fig. 2). The basal levels of GLP-1 of the control, HFS, HiFat, and HiSuc groups were 15.0 ± 1.9, 13.7 ± 3.7, 11.8 ± 2.9, and 11.9 ± 2.7, respectively. Postprandial GLP-1 levels at 15 min were significantly higher in all treatment groups (HFS, HiFat, and HiSuc: 13.0 ± 3.3 - 14.1 ± 4.4 pM; $P < 0.05$) compared with the basal level (Fig. 3C). TR on postprandial GLP-1 secretion were also detected by two-way repeated measure ANOVA in both MTTs (*P* = 0.031 and 0.003, respectively; Figs. 2C and 3C). Although significant differences were not detected, ΔAUC of GLP-1 in the HFS, HiFat, and HiSuc groups tended to increase when compared with the control group (*P* = 0.161; Fig. 3G).

Effect of chronic HFS, HiFat or HiSuc diet feeding on body weight gain, food intake, and fat accumulation

After receiving the test diet for 5 wk, body weight gain and total energy intake of the HiFat (232.3 ± 7.8 g; *P* = 0.021; and 2966.3 ±

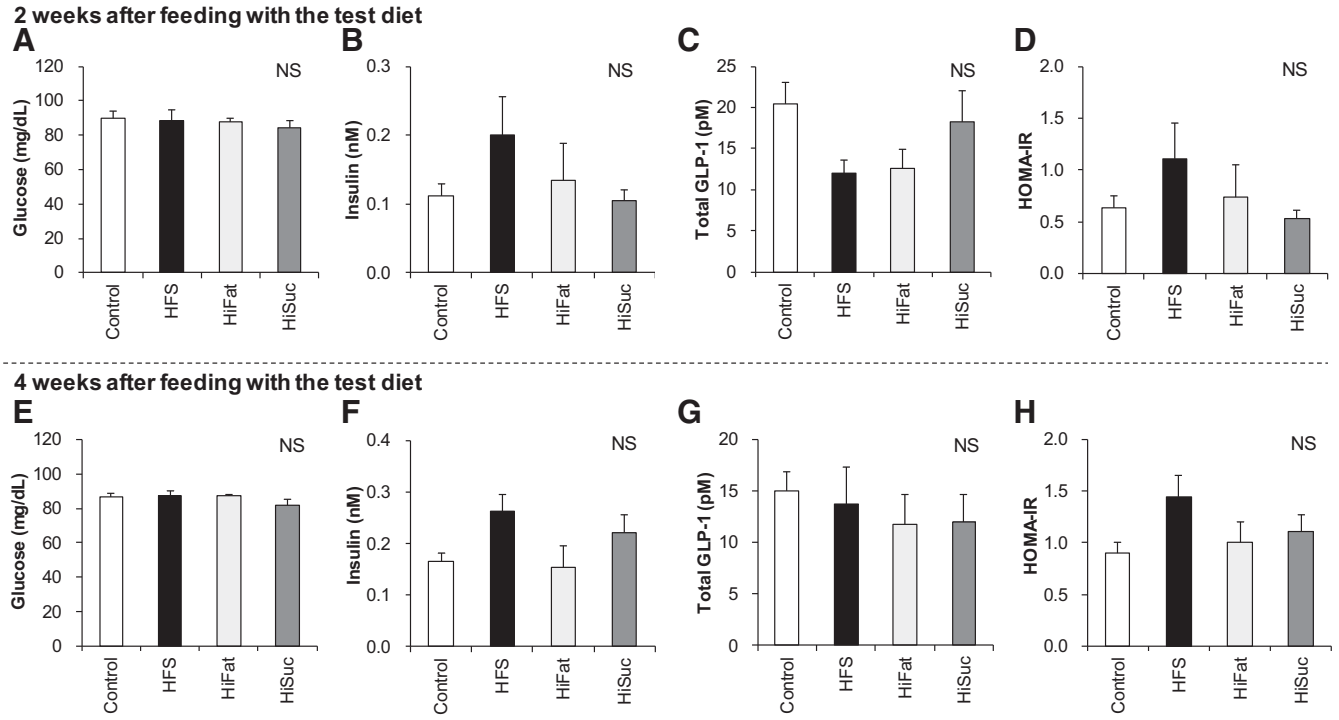

Fig. 1. Basal glucose, insulin, glucagon-like peptide-1 levels, and homeostatic model assessment of insulin resistance after feeding with the test diet for 2 and 4 wk. Rats were given the control diet, -high-fat and high-sucrose (HFS), high-fat (HiFat), or high-sucrose (HiSuc) diet for 2 and 4 wk. On the day of the experiment, blood samples were collected from the tail vein after overnight fasting (16 h) before conducting the meal tolerance test. The values were expressed as mean ± SEM for n = 4 to 9 (A-D; control = 9, HFS = 8, HiFat = 4, HiSuc = 7, E-H; control = 9, HFS = 6, HiFat = 5, HiSuc = 6) rats. NS indicates that there was no significant difference among the treatments (Tukey-Kramer's test; $P < 0.05$). HOMA-IR, homeostatic model assessment of insulin resistance.

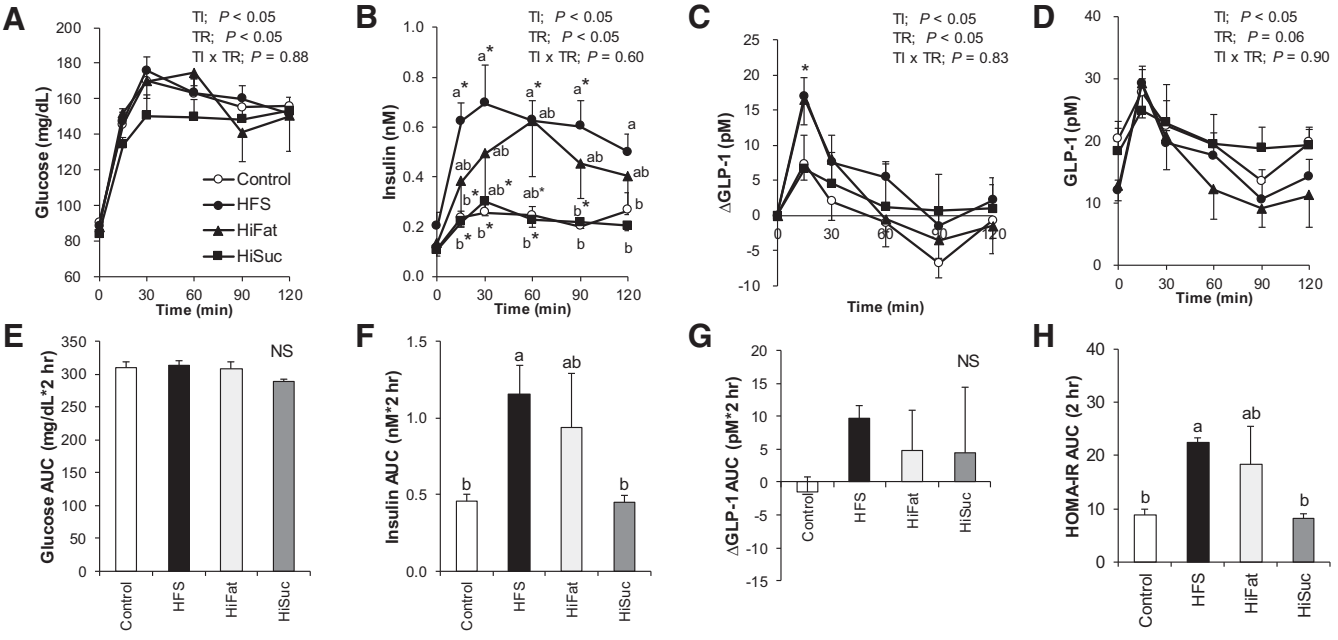

Fig. 2. Postprandial glucose, insulin, glucagon-like peptide-1 level, and homeostatic model assessment of insulin resistance during the meal tolerance test after feeding with the test diet for 2 wk. Rats were given the control, high-fat and high-sucrose (HFS), high-fat (HiFat), or high-sucrose (HiSuc) for 2 wk. Subsequently, the meal tolerance test was performed. After overnight fasting, basal blood (0 min) was taken, followed by feeding of the control diet (10 g/kg body weight) for 30 min, and blood samples were collected until 120 min. The values were expressed as mean ± SEM for n = 4 to 9 rats (control = 9, HFS = 8, HiFat = 4, HiSuc = 7). Two-way repeated measure analysis of variance *P*-values for time, treatment, and the interactions of time and treatment are shown in each panel (A, B, C). The superscripts without the same letters differed significantly between treatments ($P < 0.05$; Dunn's multiple range test). *Significant differences from the basal value (0 min) in each group ($P < 0.05$; Dunn's with control). NS indicates that there was no significant difference among the treatments. AUC, area under the curve; HOMA-IR, homeostatic model assessment of insulin resistance; TI, time; TR, treatment; TI x TR, interaction of treatment and time.

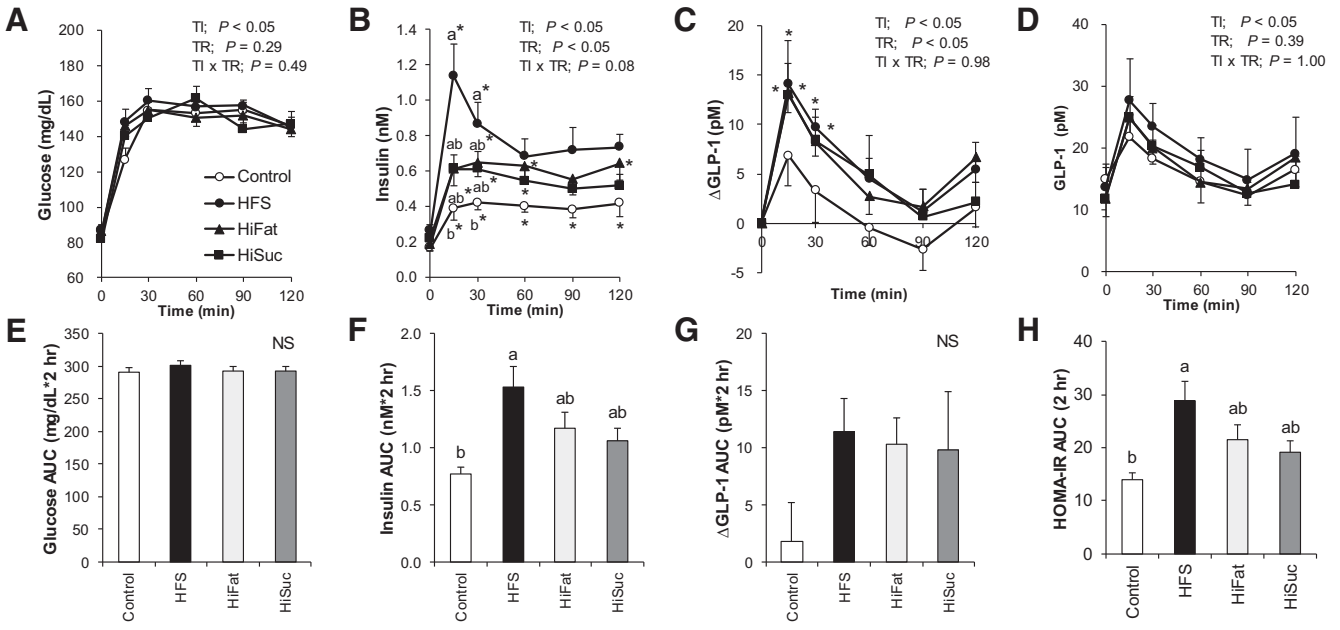

Fig. 3. Postprandial glucose, insulin, glucagon-like peptide-1 level, and homeostatic model assessment of insulin resistance during the meal tolerance test after feeding with the test diet for 4 wk. Rats were given the control, high-fat and high-sucrose (HFS), high-fat (HiFat), or high-sucrose (HiSuc) for 4 wk. Subsequently, the meal tolerance test was performed. After overnight fasting, basal blood (0 min) was taken, followed by feeding of the control diet (10 g/kg body weight) for 30 min, and blood samples were collected until 120 min. The values were expressed as mean ± SEM for n = 5 to 9 (control = 9, HFS = 6, HiFat = 5, HiSuc = 6) rats. Two-way repeated measure analysis of variance *P*-values for time, treatment, and interactions of time and treatment are shown in each panel (A, B, C). The superscripts without the same letters differed significantly between treatments ($P < 0.05$; Dunn's multiple range test). * Significant differences from the basal value (0 min) in each group ($P < 0.05$; Dunn's with control). NS indicates that there was no significant difference among the treatments. AUC, area under the curve; HOMA-IR, homeostatic model assessment of insulin resistance; TI, time; TR, treatment, TI x TR, interaction of time and treatment.

56.8 kcal; $P = 0.039$) and HFS groups (228.0 ± 7.8 g; $P = 0.039$; and 2958.6 ± 60.3 kcal; $P = 0.032$) were significantly higher than those of the control group (200.7 ±5.4 g; and 2736.6 ± 61.7 kcal; Table 2). Epididymal adipose tissue weight in the HFS group (9.7 ± 0.5 g; $P = 0.040$) was significantly higher than in the control group (7.5 ± 0.6 g). The HiSuc group showed similar values for these parameters as those in the control group.

Peptide hormones, cholesterol, and triacylglycerol levels in the portal vein after feeding test diet for 5 wk

On the final day of the experiment, blood samples were collected from the portal vein after overnight fasting. The results showed that the HFS group had the highest values for the glucose (107.3 ± 3.9 mg/dL; $P = 0.030$), insulin (1.38 ± 0.20 nM; $P = 0.069$), total cholesterol (35.9 ± 3.5 mg/dL; $P = 0.023$), and triacylglycerol levels (76.1 ± 3.1 mg/dL; $P = 0.028$; Fig. 4). The HiFat group overall showed similar results, but slightly lower values for these parameters, compared with the HFS group. The HiSuc group had results similar to the control group. The GLP-1 levels in the HFS (69.3 ± 9.0 pM; $P = 0.325$) and HiFat (61.3 ± 8.6 pM; $P = 0.812$) groups were slightly higher than the control group (51.8 ± 6.2 pM), but without significant differences (Fig. 4C).

GLP-1 content in intestinal tissue of rats after feeding with test diet for 5 wk

In the jejunum segment, the HFS group (4.8 ± 0.4 pmol/mg protein) had a significantly higher GLP-1 content than that of the control group (3.2 ± 0.2 pmol/mg protein; $P = 0.004$), but in the other segments (ileum, cecum, and colon), significant differences were not observed (Figs. 5A, B, C, and D).

Discussion

Excess energy consumption, especially from a HiFat or HiSuc diet, is a major risk factor for the development of metabolic disorders, including obesity, insulin resistance, and type II diabetes in

Table 2
Initial body weight, body weight gain, visceral adipose tissue (mesenteric, epididymal, and retroperitoneal) weight, and energy intake after feeding test diet for 5 wk

	Control	HFS	HiFat	HiSuc
Initial weight (g)	168.7 ± 3.1 ^{NS}	169.3 ± 2.5	169.1 ± 4.3	167.7 ± 2.8
Body weight gain (g)	200.7 ±5.4 ^{b}	228.0 ± 7.8^{a}	232.3 ± 7.8 ^{a}	220.3 ± 7.9 ^{a,b}
Visceral fat (g)	25.0 ± 1.6 ^{NS}	29.7 ± 1.5	29.2 ± 2.0	23.6 ± 1.4
Mesenteric fat (g)	7.2 ± 0.5 ^{NS}	7.8 ± 0.4	7.8 ± 0.5	6.7 ± 0.4
Epididymal fat (g)	7.5 ± 0.6 ^{b,c}	9.7 ± 0.5^{a}	9.3 ± 0.8 ^{a,b}	6.9 ± 0.5 ^{c}
Retroperitoneal fat (g)	10.4 ± 0.6 ^{NS}	12.1 ± 0.7	12.1 ± 0.8	10.0 ± 0.6
Total energy intake (kcal)	2736.6 ± 61.7^{b}	2958.6 ± 60.3^{a}	2966.3 ± 56.8^{a}	2809.9 ± 40.0^{a,b}

HFS, high-fat/high-sucrose diet; HiFat, high-fat diet; HiSuc, high-sucrose diet; NS, no significant difference among the treatments.
Visceral fat weight is the sum of the mesenteric, epididymal, and retroperitoneal fat weight. The values were expressed as the mean ± SEM for n = 8 to 10 rats (control = 10; HFS = 10; HiFat = 8; HiSuc = 8). The superscripts without the same letters differed significantly between treatments ($P < 0.05$; Tukey-Kramer's test).

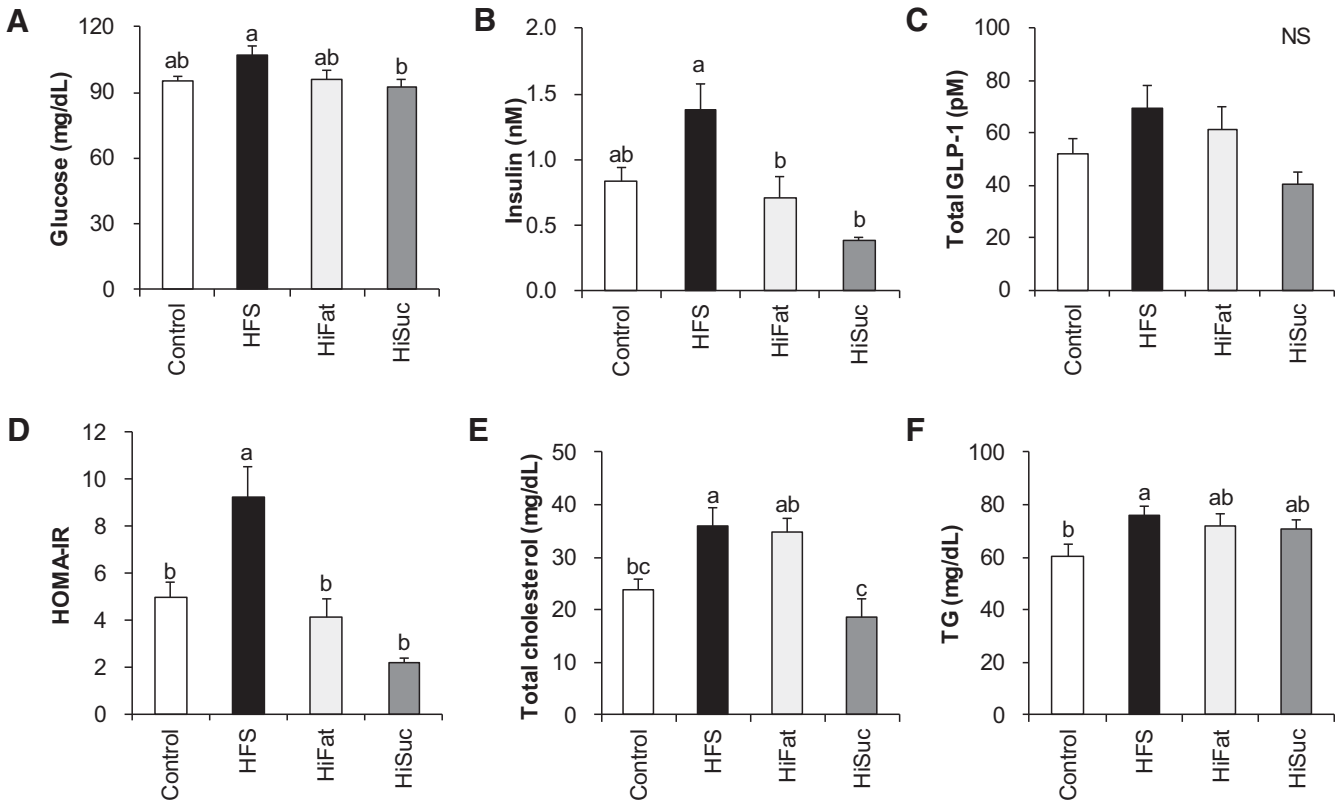

Fig. 4. Portal glucose, insulin, glucagon-like peptide-1, cholesterol, and triacylglycerol levels after feeding with the test diet for 5 wk. Rats were given the control, high-fat and high-sucrose (HFS), high-fat (HiFat), or high-sucrose (HiSuc) for 5 wk. After overnight fasting, blood samples were collected from the portal vein under sodium pentobarbital anaesthesia (50 mg/kg of body weight). The values were expressed as mean ± SEM for n = 8 to 10 (control = 10, HFS = 10, HiFat = 8, HiSuc = 8) rats. The superscripts without the same letters differed significantly between treatments ($P < 0.05$; Tukey–Kramer's test). NS indicates that there was no significant difference among the treatments. HOMA-IR, homeostatic model assessment of insulin resistance; TG, triacylglycerol.

rodents [20,24–26]. Therefore, diets that contain HiFat and/or HiSuc are commonly used to establish obesity development in animal models [24,27–29]. In this study, a HFS (30% fat and 40% sucrose), HiFat (30% fat diet), or HiSuc (40% sucrose) diet was provided to rats for a total of 5 wk to determine whether a combined HFS or individual HiFat or HiSuc diet contributed to adaptive changes in postprandial GLP-1 secretion and obesity development.

As expected, body weight gain and total energy intake in the HFS and HiFat groups were higher than in the control group, but the HiSuc group was similar to the control group. The results were also consistent with a previous report, in which chronic feeding of HFS and HiFat caused additional body weight gain and obesity development [27–33]. The consumption of a high-caloric diet directly relates to fat accumulation in various tissues and obesity development [27,29,34]. Indeed, the epididymal adipose tissue weight of the HFS and HiFat groups increased largely when compared with the HiSuc group. These results suggest that the HiFat diet rather than the HiSuc diet has a potent impact on obesity (adiposity) development in rodents. Nevertheless, an HFS diet had an intense impact on obesity development compared with a HiFat diet alone.

Currently, several methodologies, such as OGTT and MTT, are available to assess postprandial glucose and insulin responses [35,36]. However, a previous study supported the fact that MTT is more reflective of postprandial metabolic responses than OGTT [37]. In addition, we considered that the voluntary ingestion was more appropriate than enforced gavage feeding to mimic dietary exposure in normal life. Therefore, in the present study, MTT with ingestion of the standard (control) diet at a dose of 10 g/kg body weight was used to evaluate postprandial glycaemia, insulin, and GLP-1.

Unfortunately, some rats did not consume >90% weight of the provided diet (20.3–89.7% of provided diet), which could be a limitation of the present study. The data from these rats were not included because glycaemic and gut hormone responses primarily depend on the amount of food ingested. Because interpretation of the data from all rats with largely varied food consumption was difficult, we omitted the data from these rats. Lower diet consumption is assumed to result in lower GLP-1 secretion compared with rats that consumed >90% of the diet because GLP-1 secretion depends on the caloric load administered [38,39].

Nevertheless, TR (diet) was detected by two-way repeated ANOVA in MTTs. Accordingly, we consider that the oral administration of meal solution would be suitable to assess postprandial responses instead of voluntary feeding in MTT using animal models. Although difficult to control, the results obtained from the MTT with voluntary feeding should have significant meaning to understand postprandial glycaemic and gut hormone responses.

The continuous feeding of the HFS and HiFat diets for 2 wk highly affected the postprandial insulin response and slightly enhanced the postprandial GLP-1 response. Likewise, we found that the AUC of HOMA-IR was largely increased by feeding the HFS diet for 2 wk, which indicates that feeding the HFS diet immediately triggered the development of insulin resistance. Interestingly, the continuous consumption of the HiSuc diet for 4 wk illustrated that the postprandial GLP-1, insulin, and HOMA-IR parameters eventually matched those observed for the HiFat feeding group, and chronic feeding with the HiSuc diet can be concluded to

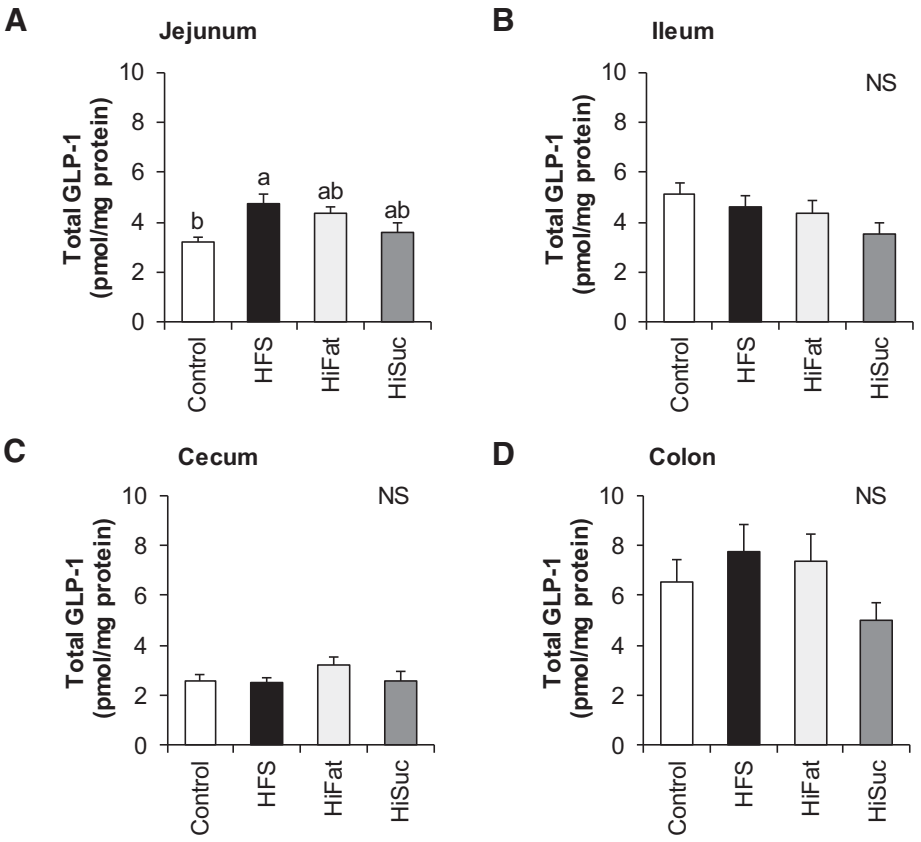

Fig. 5. Glucagon-like peptide-1 content in intestinal tissues after feeding with the test diet for 5 wk. After feeding with the test diets for 5 wk, each intestinal segment was collected: (A) Jejunum, (B) ileum, (C) cecum, and (D) colon. The values were expressed as mean ± SEM for n = 8 to 10 (control = 10, HFS = 10, HiFat = 8, HiSuc = 8) rats. The superscripts without the same letters differed significantly between treatments ($P < 0.05$; Tukey–Kramer's test). NS indicates that there was no significant difference among the treatments. GLP-1, Glucagon-like peptide-1; HFS, high-fat and high-sucrose; HiFat, high-fat; HiSuc, high-sucrose.

gradually increase postprandial insulin response and HOMA-IR index. These findings are supported by the results of a previous study that demonstrated that the HiFat diet caused severe metabolic dysfunction faster than the HiSuc diet [24].

After feeding with the test diet for 2 wk, the HFS feeding group showed a significant elevation of the postprandial GLP-1 level, but the other groups did not. All rats were given the identical control diet for the MTT; therefore, differences in the GLP-1 response should be attributed to the differences in the rat phenotype. In addition, basal GLP-1 levels did not differ significantly throughout the experimental period. One of the limitations of the present study is that significant differences were not detected in absolute GLP-1 values between treatments (Figs. 2D and 3D). However, observations of incremental GLP-1 (ΔGLP-1) have shown that postprandial GLP-1 responses differed between the groups, as assessed by two-way repeated measure ANOVA. These results suggest that the sensitivity of GLP-1-producing L cells to luminal nutrients (e.g., protein, carbohydrates, or fatty acids) was enhanced by the chronic feeding of the HFS diet.

The HiFat and HiSuc groups also had a similar response after receiving the test diet for 4 wk, which suggests that the ingestion of excessive fat and sucrose rapidly enhances the sensitivity of GLP-1-producing cells to luminal nutrients rather than individual fat or sucrose alone. GLP-1 is secreted in response to macronutrient ingestion (i.e., proteins, carbohydrates, and triacylglycerols) [8,40,41], and we used a control diet that contained all nutrients in the present study. Therefore, the specific diet components that contributed to the enhanced postprandial GLP-1 secretion in obese animals were not identified. An investigation of nutrients that enhance the sensitivity of L-cells under diet-induced obesity would be of interest.

To date, whether GLP-1 is increased or diminished during obesity development remains controversial. In this study, we found that postprandial GLP-1 levels were slightly increased in the HFS and HiFat groups, which is consistent with the results of previous studies [17,31,32]. In contrast, other studies have demonstrated that the chronic feeding of a HiFat diet diminished the GLP-1 secretion response to oral glucose and impaired the function of GLP-1-producing L-cells [19,42]. The different results observed by each research group might be due to differences in the experimental design, such as the treatment period, diet composition, and rodent species.

From the MTT experiments, postprandial glycaemic responses were apparently unchanged by the chronic feeding of the HiFat or HiSuc diets throughout the experimental period, but postprandial insulin and GLP-1 secretion increased progressively. Because significant differences were not detected in absolute GLP-1 levels among the treatment groups, we speculated that the incremental GLP-1 (ΔGLP-1) levels played an important role in blood glucose regulation through enhanced insulin secretion. This may be partially explained by the increased GLP-1 response as observed.

In addition, a recent study suggested that treatment with GLP-1 receptor antagonist exendin (9-39) reduced body weight gain in rats that were fed a HiFat diet without affecting food intake compared with untreated rats, which suggests that exendin (9-39) increased energy expenditures [43]. Moreover, increasing GLP-1

secretion in HiFat-fed rats lead to hyperinsulinemia, thus promoting energy storage (decreasing energy expenditure) and contributing to body weight gain [43]. Therefore, we speculate that the HFS and HiFat groups had a higher body weight gain compared with the control group and might involve hyperinsulinemia and lowering energy expenditure.

However, previous studies have demonstrated that HiFat diet-fed rats exhibited impaired GLP-1 signaling [44] and reduced GLP-1 production [45], which possibly promoted hyperphagia and increased body weight gain. Those reports focused on intestinal GLP-1 content and signaling instead of secretion. An examination of whether postprandial GLP-1 responses increased or decreased in other diet-induced obesity models (obese-prone and -resistant rats) may be of interest. The GLP-1 content of the jejunum segment in the HFS group was higher than that of the control group, but in other intestinal segments, significant differences were not detected, which indicates that the jejunum was more prone to adapting to an obesogenic diet feeding than other intestinal segments. L-cells in the jejunum may be assumed as the major source of enhanced postprandial GLP-1 secretion based on the present results. In support of our results, postprandial GLP-1 secretion is reported to be a direct action of the luminal contents on enteroendocrine L-cells in the distal jejunum [46,47]. Although the majority of enteroendocrine L-cells are located in the distal intestine, our result suggests that L-cells in the proximal intestine play a role in the rapid secretion of GLP-1 in response to meal ingestion.

Moreover, L-cell numbers in jejunoileum (alimentary channel) are reportedly higher than in the distal gut regions (colon) after Roux-en-Y gastric bypass [48], which suggests that L-cell numbers in the upper jejunum part were adaptively increased after surgery. In addition, the Roux-en-Y gastric bypass contributed to intestinal hypertrophy in rats, which might be due to nutrient-induced stimuli in the jejunum part [48] and appears consistent with our findings that the jejunum part is prone to adapt to stimulant rather than other intestinal segments.

Conclusions

Postprandial GLP-1 secretion was elevated in rats that were continuously fed the HFS diet within 2 wk. The continuous feeding of the HiFat or HiSuc diet for 4 wk provided a similar effect on postprandial GLP-1 response, which suggests 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 normalization of postprandial glycaemia and the slowing of the establishment of glucose intolerance.

References

[1] Karimi G, Sabran MR, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, et al. The anti-obesity effects of *Lactobacillus casei* strain Shirota versus Orlistat on high fat diet-induced obese rats. Food Nutr Res 2015;59:29273.

[2] Shirai T, Shichi Y, Sato M, Tanioka Y, Furusho T, Ota T, et al. High dietary fat-induced obesity in Wistar rats and type 2 diabetes in nonobese Goto-Kakizaki rats differentially affect retinol binding protein 4 expression and vitamin A metabolism. Nutr Res 2016;36:262–70.

[3] Bueno AA, Oyama LM, de Oliveira C, Pisani LP, Ribeiro EB, Silveira VL, et al. Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3 T3-L1 cells and C57 BL/6 J mice adipose tissue. Pflugers Arch 2008;455:701–9.

[4] Kao YH, Chang HH, Lee MJ, Chen CL. Tea, obesity, and diabetes. Mol Nutr Food Res 2006;50:188–210.

[5] Sakar Y, Duca FA, Langelier B, Devime F, Blottiere H, Delorme C, et al. Impact of high-fat feeding on basic helix-loop-helix transcription factors controlling enteroendocrine cell differentiation. Int J Obes (Lond) 2014;38:1440–8.

[6] De León DD, Li C, Delson MI, Matschinsky FM, Stanley CA, Stoffers DA. Exendin-(9-39) corrects fasting hypoglycemia in SUR-$1^{-/-}$ mice by lowering camp in pancreatic β-cells and inhibiting insulin secretion. J Biol Chem 2008;283:25786–93.

[7] Carr RD, Larsen MO, Winzell MS, Jelic K, Lindgren O, Deacon CF, et al. Incretin and islet hormonal responses to fat and protein ingestion in healthy men. Am J Physiol Endocrinol Metab 2008;295:E779–84.

[8] Tolhurst G, Reimann F, Gribble FM. Nutritional regulation of glucagon-like peptide-1 secretion. J Physiol 2009;587:27–32.

[9] Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131–57.

[10] Kim YO, Schuppan D. When GLP-1 hits the liver: A novel approach for insulin resistance and NASH. Am J Physiol Gastrointest Liver Physiol 2012;302:G759–61.

[11] Smushkin G, Sathananthan A, Man CD, Zinsmeister AR, Camilleri M, Cobelli C, et al. Defects in GLP-1 response to an oral challenge do not play a significant role in the pathogenesis of prediabetes. J Clin Endocrinol Metab 2012;97:589–98.

[12] Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: What is up, what is down? Diabetologia 2011;54:10–8.

[13] Alssema M, Rijkelijkhuizen JM, Holst JJ, Teerlink T, Scheffer PG, Eekhoff EM, et al. Preserved GLP-1 and exaggerated GIP secretion in type 2 diabetes and relationships with triglycerides and ALT. Eur J Endocrinol 2013;169:421–30.

[14] Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: Cause or consequence? Gut 1996;38:916–9.

[15] Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, et al. Determinants of the impaired secretion of glucagon- like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab 2001;86:3717–23.

[16] Calanna S, Christensen M, Holst JJ, Laferrère B, Gluud LL, Vilsbøll T, et al. Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: Systematic review and meta-analyses of clinical studies. Diabetologia 2013;56:965–72.

[17] Nakajima S, Hira T, Hara H. Postprandial glucagon-like peptide-1 secretion is increased during the progression of glucose intolerance and obesity in high-fat/high-sucrose diet-fed rats. Br J Nutr 2015;113:1477–88.

[18] Wang F, Yoder SM, Yang Q, Kohan AB, Kindel TL, Wang J, et al. Chronic high-fat feeding increases GIP and GLP-1 secretion without altering body weight. Am J Physiol Gastrointest Liver Physiol 2015;309:G807–15.

[19] Gniuli D, Calcagno A, Dalla Libera L, Calvani R, Leccesi L, Caristo ME, et al. High-fat feeding stimulates endocrine, glucose-dependent insulinotropic polypeptide (GIP)-expressing cell hyperplasia in the duodenum of Wistar rats. Diabetologia 2010;53:2233–40.

[20] Zheng J, Xiao KL, Chen L, Wu C, Hu X, Zeng T, et al. Insulin sensitizers improve the GLP-1 secretion and the amount of intestinal L cells on high-fat-diet-induced catch-up growth. Nutrition 2017;39:82–91.

[21] Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76 A diet. J Nutr 1997;127:838. S–41.

[22] Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. Am J Physiol Endocrinol Metab 2008;295:E1269–76.

[23] Cani PD, Hoste S, Guiot Y, Delzenne NM. Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. Br J Nutr 2007;98:32–7.

[24] Long Z, Zhang X, Sun Q, Liu Y, Liao N, Wu H, et al. Evolution of metabolic disorder in rats fed high sucrose or high fat diet: Focus on redox state and mitochondrial function. Gen Comp Endocrinol 2015;242:92–100.

[25] Avila-Nava A, Noriega LG, Tovar AR, Granados O, Perez-Cruz C, Pedraza-Chaverri J, et al. Food combination based on a pre-Hispanic Mexican diet decreases metabolic and cognitive abnormalities and gut microbiota dysbiosis caused by a sucrose-enriched high-fat diet in rats. Mol Nutr Food Res 2017;6.

[26] Pang J, Xi C, Huang X, Cui J, Gong H, Zhang T. Effects of excess energy intake on glucose and lipid metabolism in C57 BL/6 mice. PLoS One 2016;11:e0146675.

[27] Yang ZH, Miyahara H, Takeo J, Katayama M. Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. Diabetol Metab Syndr 2012;4:32.

[28] Sato-Mito N, Suzui M, Yoshino H, Kaburagi T, Sato K. Long term effects of high fat and sucrose diets on obesity and lymphocyte proliferation in mice. J Nutr Health Aging 2009;13:602–6.

[29] Sumiyoshi M, Sakanaka M, Kimura Y. Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. J Nutr 2006;136(3):582–7.

[30] Qi Z, Xia J, Xue X, He Q, Ji L, Ding S. Long-term treatment with nicotinamide induces glucose intolerance and skeletal muscle lipotoxicity in normal chow-fed mice: Compared to diet-induced obesity. J Nutr Biochem 2016;36:31–41.

[31] Dusaulcy R, Handgraaf S, Skarupelova S, Visentin F, Vesin C, Heddad-Masson M, et al. Functional and molecular adaptations of enteroendocrine L-cells in male obese mice are associated with preservation of pancreatic alpha-cells function and prevention of hyperglycemia. Endocrinology 2016;157:3832–43.

[32] Hira T, Suto R, Kishimoto Y, Kanahori S, Hara H. Resistant maltodextrin or fructooligosaccharides promotes GLP-1 production in male rats fed a high-fat and high-sucrose diet, and partially reduces energy intake and adiposity. Eur J Nutr 2018;57(3):965–79.

[33] Hong SJ, Lee JH, Kim EJ, Yang HJ, Park JS, Hong SK. Anti-obesity and anti-diabetic effect of neoagarooligosaccharides on high-fat diet-induced obesity in mice. Mar Drugs 2017;15:90.
[34] Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutr Res Rev 2010;23:270–99.
[35] Rijkelijkhuizen JM, Girman CJ, Mari A, Alssema M, Rhodes T, Nijpels G, et al. Classical and model-based estimates of beta-cell function during a mixed meal vs. an OGTT in a population-based cohort. Diabetes Res Clin Pract 2009;83:280–8.
[36] Staten M, Kelley DE. Using oral challenge testing to assess insulin action and secretion with mathematical modeling. Diabetes 2014;63:1188–90.
[37] Brodovicz KG, Girman CJ, Simonis-Bik AM, Rijkelijkhuizen JM, Zelis M, Bunck MC, et al. Postprandial metabolic responses to mixed versus liquid meal tests in healthy men and men with type 2 diabetes. Diabetes Res Clin Pract 2011;94:449–55.
[38] Steinert RE, Beglinger C, Langhans W. Intestinal GLP-1 and satiation: From man to rodents and back. Int J Obes (Lond) 2016;40:198–205.
[39] Ma J, Pilichiewicz AN, Feinle-Bisset C, Wishart JM, Jones KL, Horowitz M, et al. Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes. Diabet Med 2012;29:604–8.
[40] Sakamoto E, Seino Y, Fukami A, Mizutani N, Tsunekawa S, Ishikawa K, et al. Ingestion of a moderate high-sucrose diet results in glucose intolerance with reduced liver glucokinase activity and impaired glucagon-like peptide-1 secretion. J Diabetes Investig 2012;3:432–40.
[41] Shannon M, Green B, Willars G, Wilson J, Matthews N, Lamb J, et al. The endocrine disrupting potential of monosodium glutamate (MSG) on secretion of the glucagon-like peptide-1 (GLP-1) gut hormone and GLP-1 receptor interaction. Toxicol Lett 2017;265:97–105.
[42] Richards P, Pais R, Habib AM, Brighton CA, Yeo GS, Reimann F, et al. High fat diet impairs the function of glucagon-like peptide-1 producing L-cells. Peptides 2015;77:21–7.
[43] Krieger JP, Langhans W, Lee SJ. Novel role of GLP-1 receptor signaling in energy expenditure during chronic high fat diet feeding in rats. Physiol Behav 2018;192:194–9.
[44] Duca FA, Katebzadeh S, Covasa M. Impaired GLP-1 signaling contributes to reduced sensitivity to duodenal nutrients in obesity-prone rats during high-fat feeding. Obesity 2015;23:2260–8.
[45] Duca FA, Swartz TD, Sakar Y, Covasa M. Decreased intestinal nutrient response in diet-induced obese rats: Role of gut peptides and nutrient receptors. Int J Obes (Lond) 2013;37:375–81.
[46] Singh AK. Glucagon-like peptide 1 and dysglycemia: Conflict in incretin science. Indian J Endocrinol Metab 2015;19:182–7.
[47] Wang X, Liu H, Chen J, Li Y, Qu S. Multiple factors related to the secretion of glucagon-like peptide-1. Int J Endocrinol 2015;2015:651757.
[48] Hansen CF, Bueter M, Theis N, Lutz T, Paulsen S, Dalbøge LS, et al. Hypertrophy dependent doubling of L-cells in Roux-en-Y gastric bypass operated rats. PLoS One 2013;8(6):e65696.