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Glucose Intolerance Induced by a High-Fat/ Low-Carbohydrate Diet in Rats

Effects Of Nonesterified Fatty Acids

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We examined the time course of effects of a high-fat/ low-carbohydrate (HF/LC) diet on the impairment of glucose tolerance in rats, clarified whether insulin secretion and sensitivity were impaired by the HF/LC diet, and investigated the relationship between the increased nonesterified fatty acids (NEFA) after HF/LC diet feeding and insulin secretion and sensitivity. We found that glucose tolerance and the postglucose-loading insulin secretion were impaired after 3 and 7 d on the HF/LC diet. The glucose intolerance was accompanied by a rise in the fasting plasma NEFA level. When stimulated with 15 mmol/L of glucose, the insulin secretion was impaired in pancreatic islets from rats fed the HF/ LC diet. Rats fed the HF/LC diet showed insulin resistance in vivo. The glucose-stimulated insulin secretion was inhibited in the islets following 24-h culture with palmitic acid. The 24-h infusion of palmitic acid decreased whole-body insulin sensitivity. In summary, at least 3 d on a HF/LC diet is needed to induce glucose intolerance in rats, and the impairment may be induced by decreased insulin secretion and sensitivity, which is related to the increase in the plasma NEFA level.

Key Words: High-fat/low-carbohydrate diet; glucose intolerance; insulin secretion; insulin sensitivity; nonesterified fatty acids.

Introduction

In the past 50 yr, the prevalence of type 2 diabetes mellitus has been increasing in Japan *(1,2)*. Westernization of dietary habits (the decrease in grain consumption and the increase in meat and milk product consumption) is thought to be one important factor contributing to the increasing incidence of diabetes mellitus *(3)*. In fact, during 1950–1995, the consumption of milk and dairy products, meat, and eggs in Japan increased about 20- (6.6–135.3 g/d), 10- (7.6–77.7 g/d), and 7-fold (5.8–42.1 g/d), respectively *(4)*. On the other hand, the consumption of rice, the principal food of the Japanese, decreased to almost half during the same period, from 333.9 to 177.5 g/d.

Feeding of a high-fat/low-carbohydrate (HF/LC) diet to laboratory animals has been confirmed to be a useful model for the study of diabetes mellitus in humans *(5–7)*. Many researchers have demonstrated that 2–7 wk of HF/LC feeding to laboratory animals induces insulin resistance *(5,6,8)* and reduces the insulin secretion from pancreatic β-cells *(7,9)*, resulting in the impairment of glucose tolerance *(7,9)*. However, whether feeding a HF/LC diet for <2 wk can cause glucose intolerance in rats has not been established yet.

Recently, Kaneko et al. *(10)* reported that a HF/LC intake in the evening meal before a glucose tolerance test impaired glucose tolerance in healthy subjects. The mechanism has not yet been clarified. They found that the impairment of glucose tolerance was accompanied by an increase in the fasting plasma nonesterified fatty acids (NEFA) concentration. This suggests that the impairment of glucose tolerance is relevant to the increased NEFA. Some intralipid infusion experiments, by elevating the unsaturated fatty acids level, provided data suggesting the inhibitory effects of NEFA on insulin secretion *(11,12)*. Zhou and Grill *(13)* reported that the glucose-induced insulin secretion of pancreatic islets is inhibited after 48 h of previous exposure to saturated or unsaturated NEFA. However, it is unclear whether 24 h of previous culture with the elevated palmitic acid (the dominating NEFA in plasma), whose concentration is similar to that in vivo, decreases glucose-mediated insulin secretion from pancreatic islets, and it is also not clarified whether infusion of palmitic acid causes insulin resistance.

In the present study, we first examined the time needed to induce impairment of glucose tolerance in rats fed an HF/ LC diet. We then examined the effects of short-term feeding of an HF/LC diet on insulin secretion from pancreatic islets and whole-body insulin sensitivity in the glucose-intolerant rats. Finally, we used palmitic acid (the dominating

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Table 1
Plasma Glucose Concentrations
Before and After Glucose Loading,
AUC Values in Rats Fed the HF/LC or Control Diet for 3 d

Group	Glucose (mmol/L) FPG	20-min	60-min	120-min	AUC (mmol/L×min)
			1-d study		
Control	6.4 ± 0.4	10.8 ± 0.8	8.0 ± 0.4	7.3 ± 0.3	233 ± 18
HF/LC	6.6 ± 0.3	11.2 ± 1.2	8.5 ± 0.9	7.7 ± 0.7	253 ± 82
			3-d study		
Control	6.7 ± 0.2	11.1 ± 0.9	8.2 ± 0.4	7.3 ± 0.5	219 ± 62
HF/LC	7.0 ± 0.3[b]	12.9 ± 1.2[b]	9.3 ± 0.9[b]	7.8 ± 0.7[a]	298 ± 70[b]
			7-d study		
Control	6.6 ± 0.3	10.6 ± 0.9	8.3 ± 0.5	7.7 ± 0.5	230 ± 37
HF/LC	7.0 ± 0.3[b]	12.3 ± 1.1[b]	10.1 ± 0.5[b]	9.3 ± 0.5[b]	371 ± 54[b]

Values are the means ± SD for 10 rats. HF/LC, high-fat/low-carbohydrate diet; Control, control diet. [a]$p < 0.05$, [b]$p < 0.01$ vs control in the same study.

Table 2
Plasma Insulin Before and After Glucose Loading,
NEFA Concentrations,and Insulinogenic Index
in Rats Fed the HF/LC or Control Diet for 3 d

Group	Insulin (pmol/L) Before	20-min	Insulinogenic index (pmol/mmol)	NEFA (mmol/L)
		1-d study		
Control	217 ± 58	607 ± 121	89 ± 21	0.64 ± 0.04
HF/LC	218 ± 32	541 ± 92	74 ± 26	0.71 ± 0.09[a]
		3-d study		
Control	226 ± 41	609 ± 81	94 ± 42	0.62 ± 0.05
HF/LC	228 ± 59	494 ± 145[a]	49 ± 28[a]	0.73 ± 0.05[a]
		7-d study		
Control	237 ± 0	619 ± 92	99 ± 28	0.58 ± 0.09
HF/LC	252 ± 59	500 ± 109[a]	47 ± 13[a]	0.69 ± 0.10[a]

Values are means ± SD for 10 rats. HF/LC, high-fat/low-carbohydrate diet; Control, control diet; [a]$p < 0.05$ vs control in the same study.

NEFA in plasma) and examined its effect on insulin secretion in vitro, and insulin sensitivity for the first time in vivo.

Results

Time Course Effects of HF/LC Diet on Glucose Intolerance

There was no significant difference in the body weight before (HF/LC vs control: 1-d study, 229 ± 6 g vs 231 ± 8 g; 3-d study, 227 ± 9 g vs 230 ± 12 g; 7-d study, 230 ± 10 g vs 233 ± 10 g) and after each regimen (HF/LC vs control: 1-d study, 229 ± 7 g vs 232 ± 9 g; 3-d study, 228 ± 10 g vs 231 ± 12 g; 7-d study, 231 ± 8 g vs 235 ± 10 g) in any of the studies.

In the 1-d study, no significant differences were observed between the HF/LC and control groups in either the fasting plasma glucose (FPG) level, any of the postload glucose levels, or the area under the plasma glucose concentration-time curve (AUC) value (Table 1).

In the 3-d study, the FPG and the postload plasma glucose levels at 20 and 60 min were significantly higher in the HF/LC group than in the control group (Table 1). The AUC value was 36% higher in HF/LC rats than control rats.

In the 7-d study, the FPG level and all the postload glucose levels (from 20 through 120 min) were significantly higher in HF/LC rats than in control rats (Table 1). Accordingly, the AUC value of the HF/LC group was 61% higher than that of the control group.

There were no significant differences in fasting plasma insulin (FPI) levels between the HF/LC and control groups in any of the three studies (Table 2). In the 1-d study, no significant difference was observed in either the 20-min postload insulin level or insulinogenic index between HF/LC and control rats. However, in the 3-d and 7-d studies, both the 20-min postload insulin level and insulinogenic index were significantly lower in HF/LC rats than in control rats. In all three studies, HF/LC rats showed an 11–19% higher plasma NEFA level than control rats (Table 2).

Effect of HF/LC Diet on Insulin Secretion In Vitro

There was no significant difference in body weight before and after each diet regimen (data not shown). Compared with the islets isolated from control rats, those isolated from HF/LC rats exhibited an 18 and a 16% decrease in glucose-stimulated insulin secretion at 8.3 and 15 mmol/L of glucose, respectively (Fig. 1).

Perifusion analysis showed that there was no significant difference in insulin release at 3.0 mmol/L of glucose between the islets from HF/LC and control rats (Fig. 2). When the glucose concentration was increased to 15 mmol/L, the glucose-stimulated insulin release was 28–65% (from 14 to 30 min) lower in HF/LC rats than in control rats. The area under the insulin time curve for the first 6 min after the change in glucose concentration was 18 ± 12 pmol/(μg of DNA·2 min) in the islets from HF/LC rats vs 40 ± 15 pmol/(μg of DNA·2 min) in the islets from control rats ($p = 0.005$). This showed that the first phase of insulin release was impaired by the HF/LC diet.

Neither islet insulin content nor islet DNA content differed between control and HF/LC rats (control vs HF/LC: islet insulin, 687 ± 127 vs 659 ± 141 nmol/μg of DNA, $n = 8$, $p = 0.68$; islet DNA, 30 ± 4.6 vs 29 ± 3.8 ng/islet, $n = 12$, $p = 0.56$).

Effect of HF/LC Diet on Insulin Sensitivity In Vivo

There was no significant difference in body weight before and after each diet regimen (data not shown). The decrease

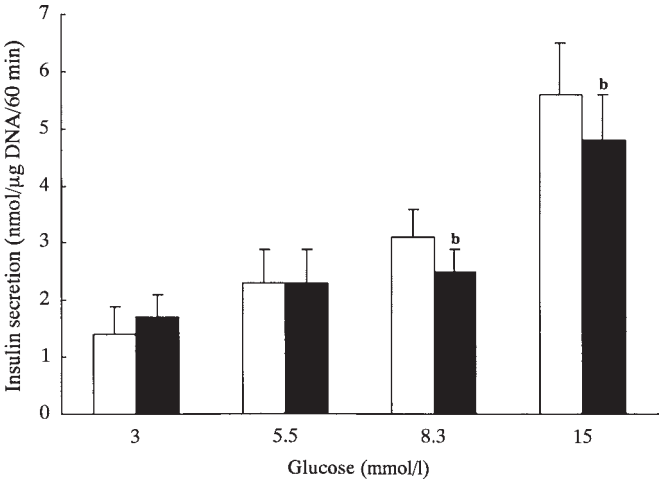

Fig. 1. Glucose-stimulated insulin secretion from freshly isolated islets from Wistar rats given HF/LC (■) or control (□) diet for 3 d. The islets were preincubated in 1.0 mL of Krebs Ringer buffer (KRB) (pH 7.4) containing 3.0 mmol/L of glucose for 60 min at 37ºC. Three size-matched islets in each culture tube were then incubated for 60 min in 1.0 mL of KRB containing glucose at 3.0, 5.5, 8.3, or 15 mmol/L. Values are expressed as means ± SD. Six separate experiments were performed with a total of 19–22 observations at each point; each observation was derived from three size-matched islets. $^{a}p < 0.05$; $^{b}p < 0.01$ vs control.

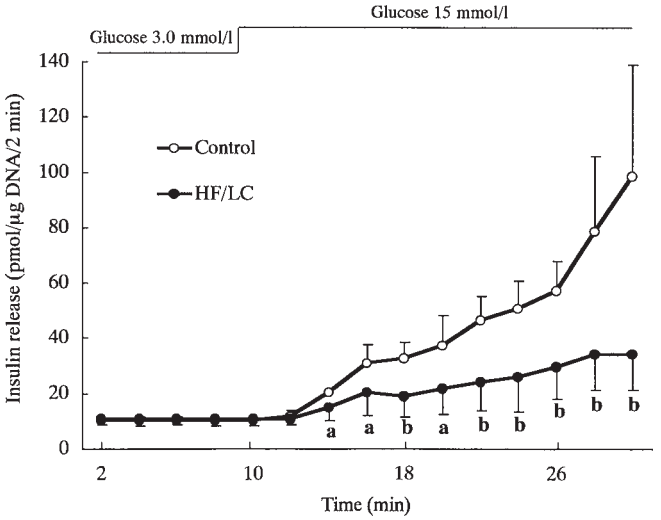

Fig. 2. Glucose-stimulated insulin release from freshly isolated perifused islets separated from Wistar rats given HF/LC (●) or control (○) diet for 3 d. The islets were preincubated in KRB (pH 7.4) containing 3.0 mmol/L of glucose for 60 min at 37ºC. Twenty-five islets in each chamber were then perifused for 30 min in KRB containing 3.0 mmol/L of glucose and changed to a medium containing 15 mmol/L of glucose, as indicated. Values are expressed as means ± SD. Six separate experiments were performed; in each series, 25 islets were perifused. $^{a}p < 0.05$; $^{b}p < 0.01$ vs control.

in the plasma glucose level after insulin injection differed between HF/LC and control rats (Fig. 3). The area above the glucose concentration time curve (AAC) value was significantly lower in HF/LC rats (57 ± 13 mmol/[L·min]) than in control rats (73 ± 14 mmol/[L·min]) ($p = 0.02$).

Effect of Palmitic Acid on Insulin Secretion In Vitro

There was no significant difference in glucose-stimulated insulin secretion between islets cultured with and without ethanol (Fig. 4A). Neither 0.4 nor 0.8 mmol/L of palmitic acid affected the insulin secretion in response to 3.0 mmol/L of glucose (Fig. 4B). However, when the islets were stimulated with 15 mmol/L of glucose, both 0.4 and 0.8 mmol/L of palmitic acid decreased the insulin secretion by 11 and 27%, respectively. Palmitic acid caused no change in either islet insulin or DNA content (data not shown).

Insulin Sensitivity after 24-h Infusion of Palmitic Acid

The fasting plasma NEFA and FPI levels were increased by about 63 and 26%, respectively, by the palmitic acid infusion, which also increased the FPG level slightly but significantly (Table 3). No significant difference was observed in body weight between palmitic acid–treated (235 ± 10 g) and palmitic acid–nontreated rats (233 ± 6 g). There was no significant difference in body weight before (232 ± 7 g) and after palmitic acid infusion.

In the intravenous insulin tolerance test (IVITT), the insulin injection (from 7 to 30 min) decreased the plasma glucose level more slowly and to a lesser extent in the palmitic acid–treated than in nontreated rats (Fig. 5). The AAC

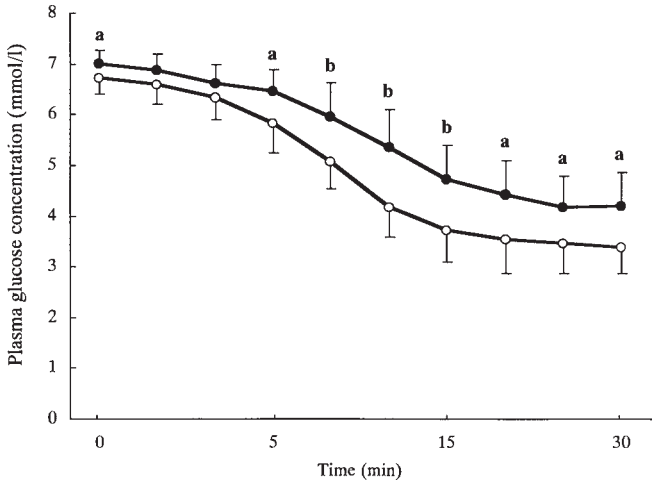

Fig. 3. Plasma glucose concentration during iv insulin tolerance test in rats given HF/LC (●) ($n = 10$) or control (○) diet ($n = 9$) for 3 d. Insulin (0.75 U/kg) was injected through the tail vein at time zero. Values are expressed as means ± SD. $^{a}p < 0.05$; $^{b}p < 0.01$ vs control.

was significantly smaller in the palmitic acid–treated (67 ± 12 mmol/[L·min]) than nontreated rats (55 ± 8 mmol/[L·min]) ($p = 0.03$).

Discussion

In previous studies, feeding rats or mice an HF/LC diet for 3–17 wk resulted in the impairment of glucose tolerance *(14–16)*. The present study demonstrated that an HF/LC diet for 3 d clearly impaired the glucose tolerance in Wistar

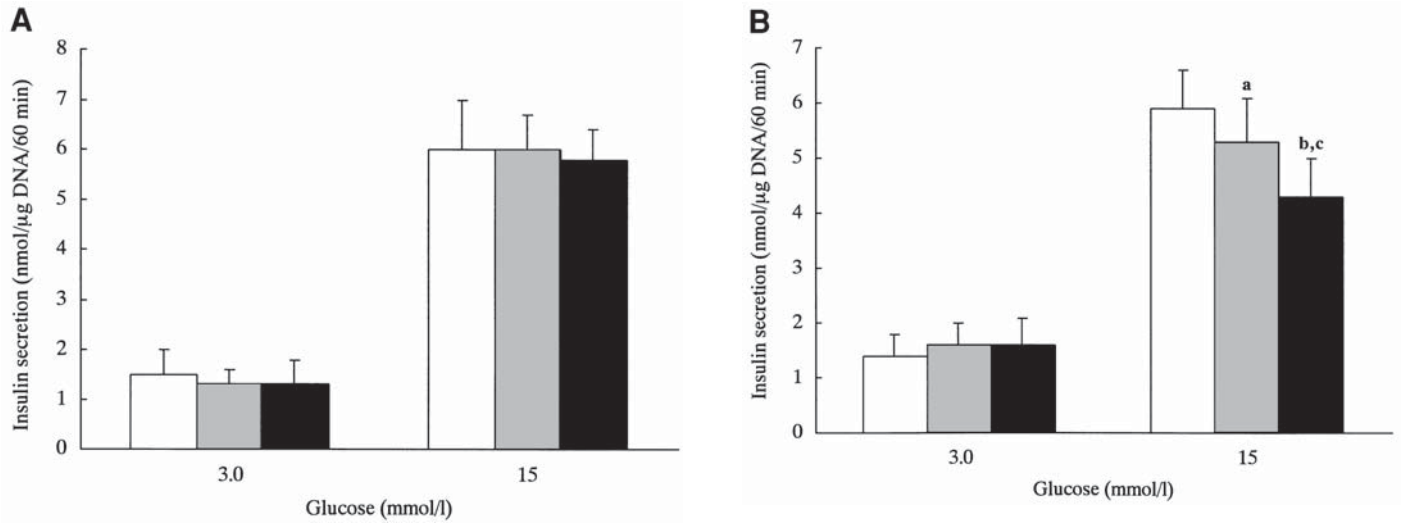

Fig. 4. Effect of palmitic acid exposure on insulin secretion from islets isolated from Wistar rats fed control diet. Islets were cultured for 24 h in Dulbecco's modified Eagle's medium (DMEM) containing both 10% fetal bovine serum (FBS) and 5.5 mmol/L of glucose without (control, □) or with 0.4 (▒) or 0.8 mmol/L (■) of palmitic acid (**B**), and without (no addition, □) or with ethanol (0.4 or 0.8%) (**A**). The islets were then preincubated in KRB (pH 7.4) containing 3.0 mmol/L of glucose for 60 min at 37°C. Three size-matched islets in each culture tube were then incubated for 60 min in 1.0 mL of KRB containing glucose (3.0 or 15 mmol/L). Values are expressed as means ± SD. Control: islets cultured with or without ethanol (0.4 or 0.8%). There was no difference in glucose-induced insulin secretion of islets cultured in DMEM with or without ethanol. $^{a}p < 0.05$; $^{b}p < 0.01$ vs control; $^{c}p < 0.01$ vs 0.4 mmol/L of palmitate.

Table 3
Fasting Plasma NEFA, Glucose, and Insulin Concentrations in Rats Infused of 154 mmol/L NaCl (NaCl), 3% BSA (BSA) or 1.0 mmol/L Palmitic Acid (PA-Treated)

	NaCl	BSA	(PA-nontreated)	PA-treated
NEFA (mmol/L)	0.55 ± 0.08	0.57 ± 0.08	(0.56 ± 0.07)	0.91 ± 0.09^{b}
Glucose (mmol/L)	6.6 ± 0.2	6.8 ± 0.2	(6.7 ± 0.2)	7.0 ± 0.2^{a}
Insulin (pmol/L)	130 ± 20	133 ± 15	(131 ± 16)	165 ± 38^{a}

Values are means ± SD. Since there was no significant difference in fasting plasma NEFA, glucose, and insulin concentrations between NaCl- and BSA-infused rats, for clarity of presentation, we combined the two groups into one group, i.e., PA-nontreated. $^{a}p < 0.05$ vs PA-nontreated; $^{b}p < 0.01$ vs PA-nontreated.

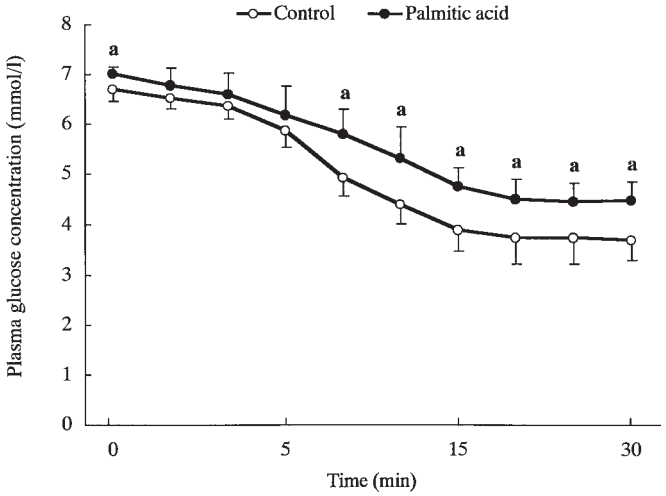

Fig. 5. Effect of palmitic acid (1.0 mmol/L) infusion for 24 h on insulin sensitivity during iv insulin tolerance test in rats. Insulin (0.75 U/kg) was injected through the catheter inserted into the jugular vein at time zero. Values are expressed as means ± SD for 10 rats. $^{a}p < 0.01$ vs palmitic acid–nontreated rats (○); palmitic acid–treated rats (●).

rats (Table 1). To our knowledge, this is the first study demonstrating this.

Rats fed an HF/LC diet for 1 d ($n = 10$) had a higher but not significant increase in the FPG level, postload plasma glucose levels, AUC value, and a lower but not significant decrease in the postload plasma insulin level. Further study is needed to document whether feeding such a diet for 1 d impairs glucose tolerance.

There was no significant difference in the FPI level between HF/LC and control rats (Table 2). Ahren et al. *(7)*, however, reported that 2- to 4-wk feeding of Sprague-Dawley rats with an HF/LC diet showed no significant increase in the FPI level, whereas 8-wk feeding of the same diet induced an evident hyperinsulinemia. This suggests that a longer time may be required to induce hyperinsulinemia using an HF/LC diet.

Ahren et al. *(7)* reported that HF/LC diet feeding for >4 wk impairs insulin secretion in response to glucose in Sprague-Dawley rats. They also showed that 2-wk feeding of the same diet decreased the first phase of insulin release

from the pancreatic islets. In the present study, we found that 3-d feeding of an HF/LC diet produced almost the same result as theirs. In the intraperitoneal glucose tolerance test (IPGTT), HF/LC diet feeding for 3 d decreased the insulin response to glucose stimulation, as revealed by the decreased 20-min postload plasma insulin level and insulinogenic index (Table 2). The dietary manipulation also decreased the glucose-stimulated insulin secretion from the pancreatic islets of Langerhans in vitro. This suggests that the impairment of glucose tolerance by 3-d HF/LC diet feeding is associated, at least in part, with decreased glucose-induced insulin secretion from the islets.

The present study demonstrated that the impairment of glucose tolerance by 3-d HF/LC diet feeding was accompanied by an increased fasting plasma NEFA level (Tables 1 and 2). Randle *(17,18)* and Randle et al. *(19)* proposed that an increase in the NEFA level inhibits glucose oxidation, stimulates hepatic glucose production, and inhibits secretion of insulin by β-cells in the pancreatic islets in response to glucose. It has been reported that 48-h exposure of islets to NEFA inhibits glucose-induced insulin secretion *(13)*. In the present study, even a 24-h exposure of islets to palmitic acid reduced the glucose-stimulated insulin secretion (Fig. 4B). Taken together, these findings indicate that an increased NEFA level reduces insulin secretion from pancreatic islets.

In the present study, the islet insulin content and the islet DNA content (an index of β-cell numbers in the pancreatic islets) were not affected by 3-d HF/LC diet feeding or by 24-h exposure of islets to palmitic acid. By contrast, Zhou and Grill *(13)* reported that 48-h culture of pancreatic islets with NEFA reduced the insulin content. Culture of islets with NEFA for <48 h may not reduce the content. Further studies are needed.

Short-time IVITT demonstrated that only 3-d HF/LC diet feeding impaired whole-body insulin sensitivity in rats (Fig. 3). Moreover, we first reported here that the increased NEFA level in the plasma by 24-h infusion of palmitic acid deteriorated insulin sensitivity (Fig. 5). This finding suggests that an elevated NEFA level decreases whole-body insulin sensitivity.

Akiyama et al. *(16)* reported that overfeeding of a high-fat diet induces obesity, glucose intolerance, and insulin resistance in normal Wistar rats. Recently, Wang et al. *(20)* found that just 3 or 7 d of a highly palatable diet (33% fat, 45% carbohydrate, 22% protein) overfeeding can lead to rapid weight gain and onset of insulin resistance in Sprague-Dawley rats. In the present study, we found that even restricted HF/LC diet intake for 3 d impaired glucose tolerance and decreased glucose-stimulated insulin secretion and insulin sensitivity without body weight gain. Taken together, these data suggest that a HF/LC diet may lead to impaired glucose tolerance, decreased insulin sensitivity, and decreased glucose-mediated insulin release through excessive caloric intake as well as a change in dietary composition.

Table 4
The Composition of the Test Diets

Components[a]	Control diet	HF/LC diet
Carbohydrate	60.0 (60.0)	13.8 (10.0)
Dextrin	30.0 (30.0)	6.92 (5.0)
Maltose	30.0 (30.0)	6.92 (5.0)
Protein	24.5 (25.0)	34.6 (25.0)
Casein Na	24.5 (24.53)	33.93 (24.53)
L-Cystine	0.29 (0.29)	0.41 (0.29)
DL-Methionine	0.18 (0.18)	0.25 (0.18)
Fat	6.66 (15.0)	40.0 (65.0)
Corn oil	1.28 (2.89)	8.86 (14.4)
Olive oil	4.29 (9.65)	29.6 (48.1)
Ethyl linoleate	1.09 (2.46)	1.51 (2.46)
Mineral mixture	2.88	3.98
Vitamin mixture	0.04	0.05
Fiber	5.48	7.59
Xanthan gum	1.22	1.68
Choline bitartrate	0.21	0.29

[a]g/100 g diet with % calories in parentheses.

In conclusion, HF/LC diet feeding for 3 d impaired glucose tolerance by inhibiting insulin secretion from pancreatic islets and by decreasing whole-body insulin sensitivity. The elevation of NEFA may be associated with the glucose intolerance via the Randle cycle, which is the activation of the glucose–fatty acids cycle.

Materials and Methods

Animals

Eight-wk-old male Wistar rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were used throughout the experiments. They were kept individually in stainless steel wire-bottomed cages in an air-conditioned room (22 ± 2ºC, 55 ± 10% relative humidity) with artificial lighting from 6:00 AM to 6:00 PM. For acclimation, they were maintained on a control diet and water ad libitum for 1 wk. They were then switched to either the control or HF/LC diet. Both diets were semipurified diets (Table 4). The control diet contained 60% carbohydrates, 15% fat, and 25% protein in calories, while the HF/LC diet contained 10% carbohydrates, 65% fat, and 25% protein. The latter was prepared by replacing the carbohydrate in the control diet with an isocaloric amount of fat.

The daily calorie intake was 60 kcal/rat (pair feeding), unless otherwise specified. The food was replenished daily at 4:00 PM. Most animals consumed their daily ration by 10:00 AM the following day. The food remaining, if any, was withdrawn at this time.

The experiments were performed in accordance with the Guidelines for Animal Experiments of the Yamanashi Medical University, which concur with the US National Institutes of Health Guidelines.

Time Course Effects of HF/LC Diet on Glucose Intolerance

At 9 wk of age, 30 rats were randomly divided into three groups (10 rats in each group) to be used for the 1-, 3-, and 7-d studies. Half of the rats in each group were fed the control diet and the other half were fed the HF/LC diet for the same number of days before an IPGTT. At least 1 wk later, the rats that had been first assigned to the HF/LC diet were fed the control diet for 1, 3, and 7 d, and vice versa, and underwent a second IPGTT. All rats were kept on the control diet between the two tests.

The IPGTT was performed as follows: Around 4:00 PM on the experimental day, fasting blood was collected in hematocrit tubes from a cut on the tail vein of the rats. The rats were then administered 2.0 g/kg of glucose (20%) (Otsuka Pharmaceutical, Tokyo, Japan) intraperitoneally. In a preliminary experiment, the peaks of both glycemia and insulinemia appeared around 20 min after ip glucose loading. Hence, blood was collected from the tail vein at 20, 60, and 120 min after glucose loading. The increment in plasma glucose following the glucose load was expressed in terms of the AUC from the time when the fasting blood was drawn until the 120-min postload blood sampling, using the trapezoidal rule. An insulinogenic index, defined as the ratio of the change in circulating insulin to the change in the corresponding glycemic stimulus *(21)*, was calculated using the following equation:

$$\frac{\text{(20-min plasma insulin} - \text{FPI)}}{\text{(20-min plasma glucose} - \text{FPG)}}$$

Effect of HF/LC Diet on Insulin Secretion In Vitro

Nine-week-old rats kept on the control or HF/LC diet for 3 d were anesthetized by ip injection of 50 mg/kg of pentobarbital (sodium salt; Abbott, North Chicago, IL). Pancreatic islets were isolated from the pancreas by collagenase digestion *(22)*. In brief, the pancreas was retrogradely filled with 30 mL of Hank's balanced salt solution (glucose concentration of 5.5 mmol/L) (Gibco-BRL) supplemented with 22 mg of collagenase (183 U/mg) (Wako, Osaka, Japan). The pancreas was then removed and incubated for 30 min at 37ºC. After rinsing, the islets were separated from the remaining exocrine tissue by hand picking under a stereomicroscope.

Islets were preincubated for 60 min at 37ºC, in an atmosphere of 95% O_2 and 5% CO_2, in KRB containing 3.0 mmol/L of glucose (basal incubation solution). The buffer consisted of 120 mmol/L of NaCl, 4.8 mmol/L of KCl, 2.5 mmol/L of $CaCl_2$, 1.2 mmol/L of $MgCl_2$, 24 mmol/L of $NaHCO_3$, and 100 mg/mL of bovine serum albumin (BSA). After the preincubation, three size-matched islets were transferred into culture tubes containing 1.0 mL of KRB supplemented with glucose at different concentrations. After incubating for 60 min, 0.5 mL of the solution was collected from each tube and immediately stored at –20ºC until insulin analysis.

After the preincubation just described, groups of 25 islets were transferred to perifusion chambers. The islets were perifused at a flow rate of 1.0 mL/min at 37ºC with the KRB. After 30 min of perifusion in KRB containing 3.0 mmol/L of glucose, the glucose concentration was increased to 15 mmol/L. Samples were taken in 2-min intervals for a total of 30 min and stored at –20ºC until analysis of the insulin concentration.

The islet insulin content was measured according to the method described by Ishihara et al. *(23)*. The islet DNA content, as an indicator of cell number, was measured by the method of Labarca and Paigen *(24)*, which was modified by Hopcroft et al. *(25)*, using calf thymus DNA (Type I; Sigma, St. Louis, MO) as standard.

Effect of HF/LC Diet on Insulin Sensitivity In Vivo

IVITT was performed on 9-wk-old rats that had been kept on the control or HF/LC diet for 3 d as follows: At 4:00 PM on the experimental day, an initial blood sample was taken from the tail vein in heparinized capillary tubes to measure FPG. Porcine insulin (Sigma) was then injected into the other tail vein at 0.75 U/kg. After the injection (time = 0), blood was collected from the tail vein at 1, 3, 5, 7, 10, 15, 20, 25, and 30 min. Blood samples were immediately centrifuged to separate plasma for assaying glucose concentration. The decrease in the plasma glucose concentration was expressed by the AAC from the time when the fasting blood was drawn until the 30-min postload blood sampling, using the trapezoidal rule.

Effect of NEFA on Insulin Secretion In Vitro

Nine-week-old rats fed the control diet were anesthetized with 50 mg/kg of pentobarbital (sodium salt; Abbott). After the isolation as described earlier, islets were transferred to Petri dishes containing DMEM (pH 7.4, glucose concentration of 5.5 mmol/L) (Gibco-BRL) supplemented with 10% heat-inactivated FBS (Gibco-BRL), 100 U/mL of penicillin (Sigma), and 0.1 mg/mL of streptomycin (Sigma). Then islets were cultured for 24 h at 37ºC in an atmosphere of 5% CO_2/95% air with or without palmitic acid, which is a dominating NEFA in the plasma. Palmitic acid (sodium salt; Sigma) was dissolved in 99% ethanol. The ethanol solution was mixed with the same volume of 154 mmol/L of NaCl, which was then added to the DMEM. The final concentration of NEFA in the medium used was 0, 0.4, or 0.8 mmol/L. The final ethanol concentration was 0, 0.4, or 0.8% (v/v) according to the palmitic acid concentration used, respectively.

After rinsing, the islets were preincubated for 60 min for their recovery. Three size-matched islets were challenged with 15 mmol/L of glucose in KRB solution at 37ºC for 60 min. Then, 0.5 mL of the solution was collected and immediately stored at –20ºC until insulin analysis.

Effect of NEFA on Insulin Sensitivity In Vivo

Nine-week-old rats kept on the control diet underwent jugular catheterization after ip injection of pentobarbital (50 mg/kg). Jugular catheterization was performed with a sterile Disposal Swivel Kit (DS-10; Bio Research, Tokyo, Japan). The Swivel Kit protected the infusion tube from being bitten and pulled out by the rats and gave complete freedom of movement to the rats while in the cages. Catheters were kept patent by continuous infusion of 154 mmol/L of NaCl (Otsuka) at a rate of 1.0 mL/h. A 3- to 5-d recovery period followed insertion of the catheter. During this period, the animals gained body weight (from 228 ± 7 to 234 ± 8 g; $n = 20$, $p = 0.04$).

At 4:00 PM on the day before IVITT, three groups of rats were infused with one of the following solutions for 24 h: 1.0 mmol/L of palmitic acid, 154 mmol/L of NaCl, or 154 mmol/L of NaCl containing 3% fatty acid–free BSA (BSA group). Palmitic acid (sodium salt) was dissolved in 99% ethanol and diluted in 154 mmol/L of NaCl solution containing fatty acid–free BSA (the final concentrations of ethanol and BSA were 1 and 3%, respectively). The high albumin concentration was used to mimic that of the serum. In the NaCl and BSA infusates, ethanol was added at a final concentration of 1% for comparison. The infusion rate, controlled by a syringe pump, was 1.0 mL/h. Food intake by rats during infusion with palmitic acid, NaCl, and BSA were 50 ± 8, 52 ± 7, and 51 ± 10 kcal/rat, respectively (no significant difference in food intake; $n = 7$, $p > 0.05$).

At 4:00 PM on the next day, an initial blood sample was taken from the tail vein in heparinized capillary tubes to measure FPG, FPI, and plasma NEFA levels. Porcine insulin (0.75 U/kg) was then injected through the jugular catheter, and the blood was collected at 1, 3, 5, 7, 10, 15, 20, and 30 min after the injection.

Biochemical Analyses

NEFA and glucose concentrations were measured with an NEFA C-Test (Wako) and a Glucose CII-Test (Wako), using a spectrophotometer (Clinical Spectrophotometer 7010 with an X-Y Autosampler; Hitachi). The insulin concentration was determined with an Insulin ELISA Kit from Morinaga Biochemistry (Yokohama, Japan) using rat insulin as standard with a microplate spectrophotometer system (SPECTRAmax 340 with SOFTmax PRO version 2.1 software; Molecular Devices, Sunnyvale, CA). The intra- and interassay coefficients of variation for this insulin assay were each <10%, with the minimum detectable concentration being 50 pg/mL.

Statistical Analyses

One- or two-way analysis of variance and Fisher's PLSD test or student's *t*-test (StatView 5.0; Abacus Concepts, Berkeley, CA) were used when there was a significant difference among the groups, as appropriate. The 0.05 level of probability was used as the criterion of significance.

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