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The Development of Diabetes Mellitus in Wistar Rats Kept on a High-Fat/Low-Carbohydrate Diet for Long Periods

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The present study was performed to determine the effect of long-term feeding with a high-fat/low-carbohydrate (HF/LC) diet on the onset of type-2 diabetes mellitus in normal rats. Male Wistar Imamichi rats were kept on a Control (carbohydrate 60%, fat 15%) or HF/LC (carbohydrate 10%, fat 65%) diet for 16 mo. An intraperitoneal glucose tolerance test was performed once every 2 mo. Glucose tolerance was impaired 2 mo after the start of HF/LC diet feeding, accompanied by a decrease in the insulinogenic index. Along with time of HF/LC diet feeding, the glucose tolerance was further deteriorated with more serious impairment of insulin secretion and sensitivity. At the end of the experiment, 15 of 18 rats in the HF/LC group were diabetic, whereas only 4 of 17 rats in the Control group were diabetic. The present results demonstrate that long-term feeding with a HF/LC diet decreases the secretion and sensitivity of insulin, and induces diabetes mellitus in rats. Furthermore, long-term feeding with such a diet may produce adverse effects on the blood plasma lipid profile, with elevated levels of triglycerides, non-esterified fatty acids, total cholesterol, and reduced levels of high density protein cholesterol in the plasma.

Key Words: Diabetes mellitus; high-fat/low-carbohydrate diet; glucose intolerance; insulin secretion; insulin resistance; non-esterified fatty acids.

Introduction

The prevalence of type-2 diabetes mellitus (DM) has increased in Japan *(1,2)* over the last 50 yr. Kawate et al. suggested this was attributable to the westernization of the population's dietary habits, i.e., the decrease in grain consumption and the increase in meat and milk product consumption *(3)*. However, this suggestion is based only on a limited number of epidemiological studies performed on Japanese immigrants *(3,4)*. It is not enough to support this hypothesis.

Kaneko et al. *(5)* reported that a high-fat/low-carbohydrate intake in the evening meal before a glucose tolerance test impaired glucose tolerance in healthy subjects. Kaneko et al. *(6)* also demonstrated by a long-term experiment that isocaloric high-fat/low-carbohydrate (HF/LC) diet feeding aggravated diabetes mellitus in genetically diabetic rats. However, it was not clarified whether long-term HF/LC diet feeding induces diabetes mellitus in normal rats.

Feeding of laboratory animals with a HF/LC diet has been shown to be a useful model to study the effects of dietary fat on diabetes mellitus in humans. A number of investigators have demonstrated that 2–7 wk of high-fat feeding to laboratory animals induces insulin resistance *(7–9)*, reduces the insulin secretion of β cells, and results in the development of glucose intolerance *(10,11)*. Previously, we *(12)* demonstrated that feeding with a HF/LC diet for 3 d induces impaired glucose tolerance, which was accompanied by the deterioration of glucose-stimulated insulin secretion from β cells and whole body insulin sensitivity in Wistar rats. However, Chalkey's group reported that administration of HF/LC diet to rats for 10 mo induced insulin resistance but did not develop into diabetes mellitus *(13)*. Therefore, we observed whether or not feeding with a HF/LC diet for long period induced DM in rats.

In the present study, we fed normal rats a HF/LC diet for 16 mo, and examined the long-term effect of this diet on the onset of DM.

Results

Body Weight

In the Control group, the body weight increased gradually during the first 6 mo (Fig.1). A reduction in the rate of body weight gain was observed from 6 to 14 mo. The body weight decreased at 16 mo, yet the extent of the changes was smaller compared with that of the HF/LC group.

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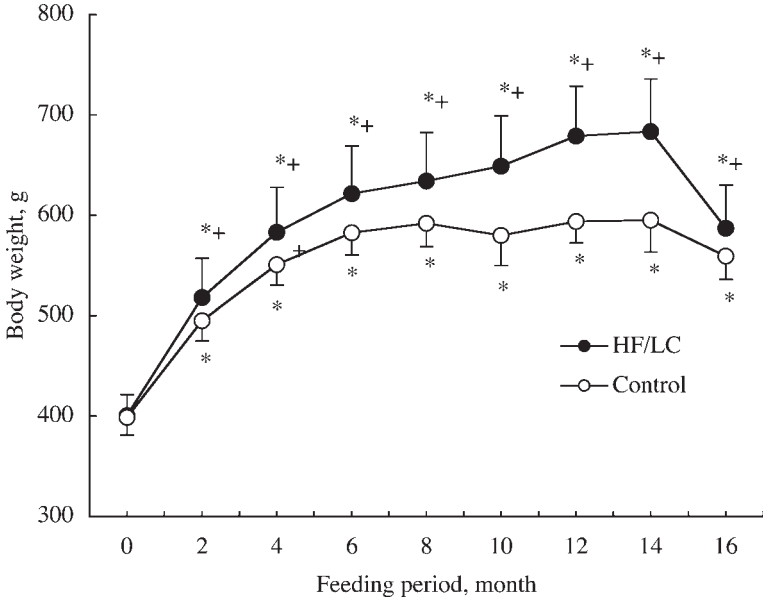

Fig. 1. Long-term effect of the high-fat/low-carbohydrate diet feeding on body weight in rats. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

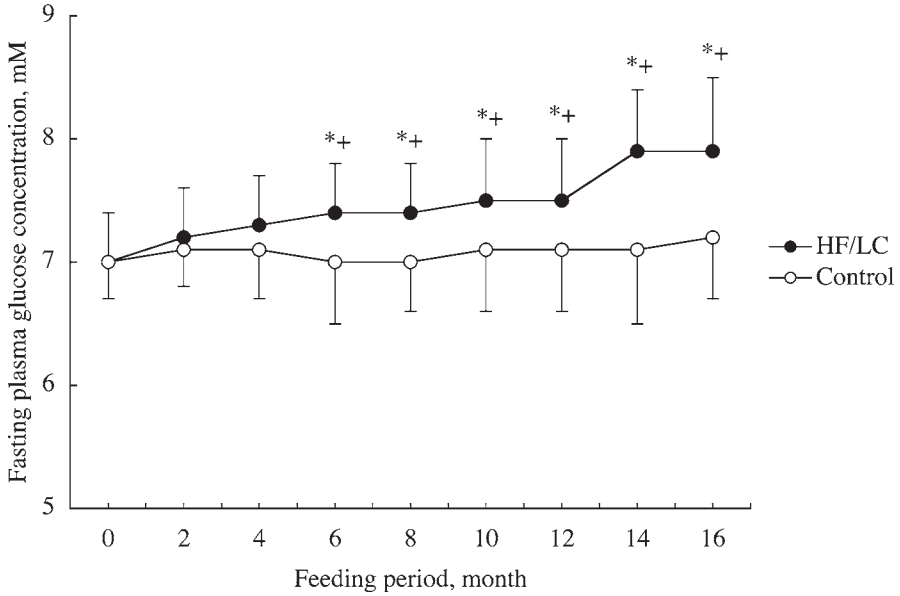

Fig. 2. Time course effects of the high-fat/low-carbohydrate diet on the fasting plasma glucose concentration. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

From 4 mo, the body weight in HF/LC group maintained a higher level than the Control group (Fig. 1), but it decreased after 14 mo; it especially decreased sharply at 16 mo.

Plasma Glucose Concentration in Intraperitoneal Glucose Tolerance Test (IPGTT)

In the Control group, there was no significant change in the fasting plasma glucose (FPG) level (7.0 ± 0.3 to 7.2 ± 0.5 m*M*) during 16-mo experimental period (Fig. 2). In the HF/LC group, the FPG level increased significantly from 6 mo of feeding (7.0 ± 0.4 to 7.4 ± 0.4 m*M*, $p < 0.05$, Fig. 2) and increased further at 14 mo (7.9 ± 0.5 m*M*) and 16 mo (7.9 ± 0.6 m*M*) of feeding ($p < 0.05$). The FPG level in the HF/LC group was significantly higher than that in the Control group after 6 mo of feeding ($p < 0.05$, Fig. 2).

Figure 3 shows the changes in the 120-min postload plasma glucose during the experiment. In the Control group, the value increased gradually from 8.6 ± 0.7 to 10.0 ± 0.6 m*M* during the first 6 mo of feeding ($p < 0.05$). From 6 to 16 mo of feeding, the 120-min postload plasma glucose

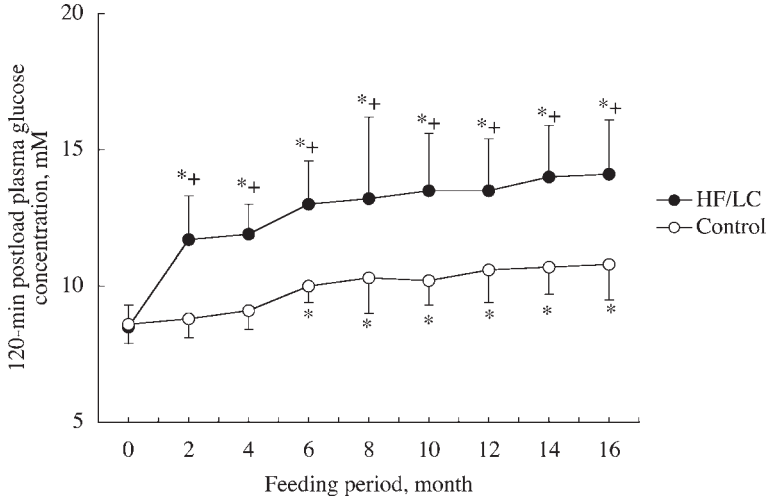

Fig. 3. Time course effects of the high-fat/low-carbohydrate diet on the 120-min postload plasma glucose concentration. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

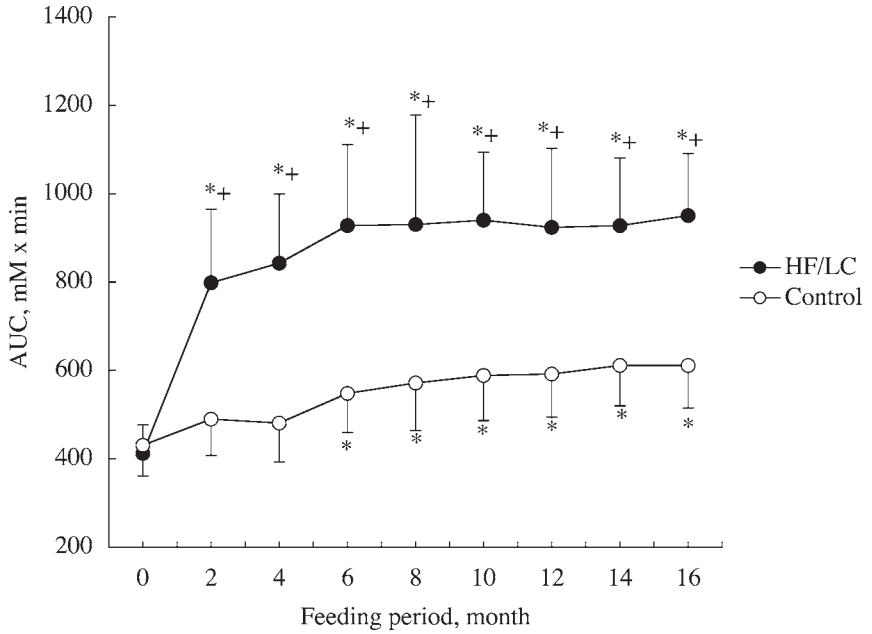

Fig. 4. Time course effects of the high-fat/low-carbohydrate diet on the AUC. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

level showed no significant changes ($p > 0.05$). In the HF/LC group, the 120-min postload plasma glucose increased significantly from 8.5 ± 0.8 to 11.7 ± 1.6 m*M* after 2 mo of feeding ($p < 0.05$, Fig. 3). The level was also significantly higher compared with the Control group at 2 mo ($p < 0.05$). Thereafter, the level remained higher level than that in the Control group and reached 14.1 ± 2.0 m*M* at the end of experiment (16 mo).

In the Control group, the area under the curve (AUC) for plasma glucose increased gradually from 431 ± 70 to 548 ± 89 m*M* × min after 6 mo of feeding (Fig. 4; $p < 0.05$). There was no significant change in the AUC from 6 to 16 mo of the experiment. In the HF/LC group, the AUC values significantly increased from 412 ± 65 to 789 ± 167 m*M* × min after 2 mo of the feeding ($p < 0.05$, Fig. 4). The value in the HF/LC group was also clearly higher than that of the Control group and maintained a higher level from 2 mo to the end of the experiment ($p < 0.05$).

To sum up the above results, the FPG, 120-min postload plasma glucose levels, and AUC were significantly higher

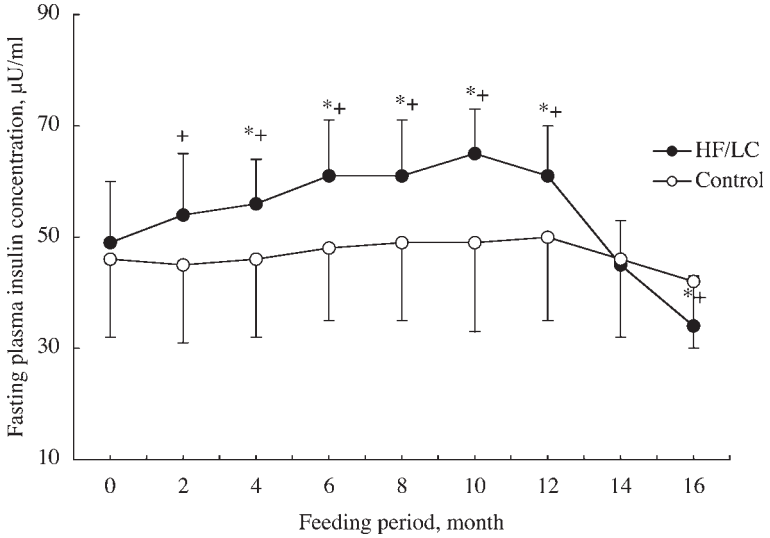

Fig. 5. Time course effects of the high-fat/low-carbohydrate diet on the fasting plasma insulin concentration. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

in the HF/LC group than those in the Control group (Figs. 2–4).

As the criteria for diabetic type are a peak plasma glucose concentration > 16.8 m*M* or 120-min postload plasma glucose concentration > 11.2 m*M (14)*, 12 rats were classified as diabetic after being fed HF/LC diet for 6 mo, while none of the rats became diabetic in the control group during the same period. At the end of the experiment (16 mo), 15 of 18 rats were diabetic in the HF/LC group, whereas only 4 of 17 rats were diabetic in the Control group. Three rats of the Control group and 2 rats of the HF/LC group were dead because of intraperitoneal glucose administration.

Plasma Insulin Level, Insulinogenic Index, and HOMA-IR

In the Control group, the fasting plasma insulin (FPI) value showed no significant changes (minimum 42 ± 12, maximum 50 ± 15 μU/mL, $p > 0.05$, Fig. 5) throughout the experiment. In the HF/LC group, the FPI value increased significantly from 49 ± 11 to 56 ± 8 μU/mL after 4 mo of feeding ($p < 0.05$, Fig. 5) and reached the peak (65 ± 8 μU/mL) at 10 mo. Then the value began to decrease from 12 mo, and was reduced to 34 ± 9 μU/mL at 16 mo of feeding ($p < 0.05$). Moreover, the FPI level in the HF/LC rats was significantly higher than that in the Control rats between 2 and 12 mo of feeding. However, the HF/LC rats had a significantly lower FPI than the Control rats at 16 mo of feeding.

Figure 6 shows changes in the 20-min postload plasma insulin level during the experiment. In the Control group, no significant changes were seen in the level during the 14 mo of feeding (minimum 71 ± 14, maximum 76 ± 16 μU/mL, $p > 0.05$). At 16 mo of feeding, the 20-min postload plasma insulin level decreased to 66 ± 14 μU/mL ($p < 0.05$). In the HF/LC group, there were no significant changes in the 20-min postload plasma insulin level from beginning to 12 mo of feeding (minimum 69 ± 10, maximum 76 ± 8 μU/mL). At 14 and 16 mo, the level decreased to 62 ± 8 and 49 ± 9 μU/mL, respectively, which were also significantly lower than the corresponding values in the Control group ($p < 0.05$).

The time course pattern of the insulinogenic index *(15)*, in the HF/LC group was completely different from that observed in the Control group (Fig. 7). In the Control group there were no significantly changes in the index during first 12 mo (4.1 ± 1.7 to 3.2 ± 1.2 mU/mmol, $p > 0.05$). However, clear decreases were observed at 14 (2.9 ± 1.4 mU/mmol) and 16 mo (3.0 ± 1.3 mU/mmol) of the experiment ($p < 0.05$). In the HF/LC group, the insulinogenic index decreased from 4.1 ± 1.6 to 1.5 ± 0.4 mU/mmol after 2 mo of feeding ($p < 0.05$, Fig. 7) and maintained the low level until the end of the experiment (1.2 ± 0.5 mU/mmol). Moreover, the index in HF/LC rats was significantly lower than that in the Control rats between 2 and 16 mo of feeding ($p < 0.05$).

Figure 8 shows changes of the "homeostasis model assessment of insulin resistance (HOMA-IR) *(16)* during the experiment. In the Control group, there was no significant difference in HOMA-IR during the 14 mo of feeding (minimum 14.8 ± 2.8, maximum 16.6 ± 3.2, $p > 0.05$). However, the HOMA-IR at 16 mo of feeding (13.9 ± 2.2) was lower than those between 6 and 12 mo of feeding ($p < 0.05$). In the HF/LC group, the value showed a gradual increase from 2 mo, reaching a peak (21.4 ± 3.6) at 10 mo of feeding. However, the HOMA-IR was decreased at 14 (15.8 ± 3.2) and 16 mo (12.3 ± 3.2) of the experiment ($p < 0.05$). From

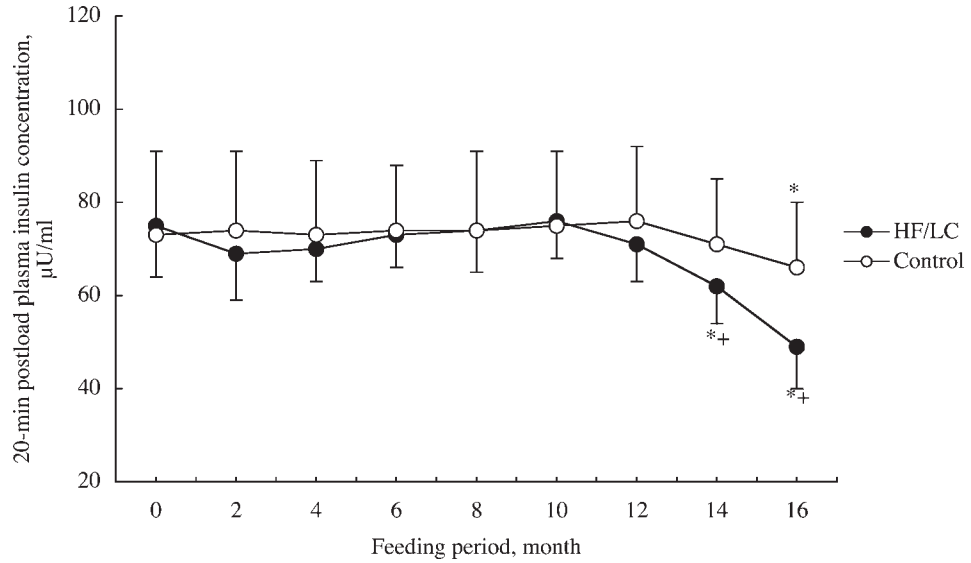

Fig. 6. Time course effects of the high-fat/low-carbohydrate diet on the 20-min postload plasma insulin concentration. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

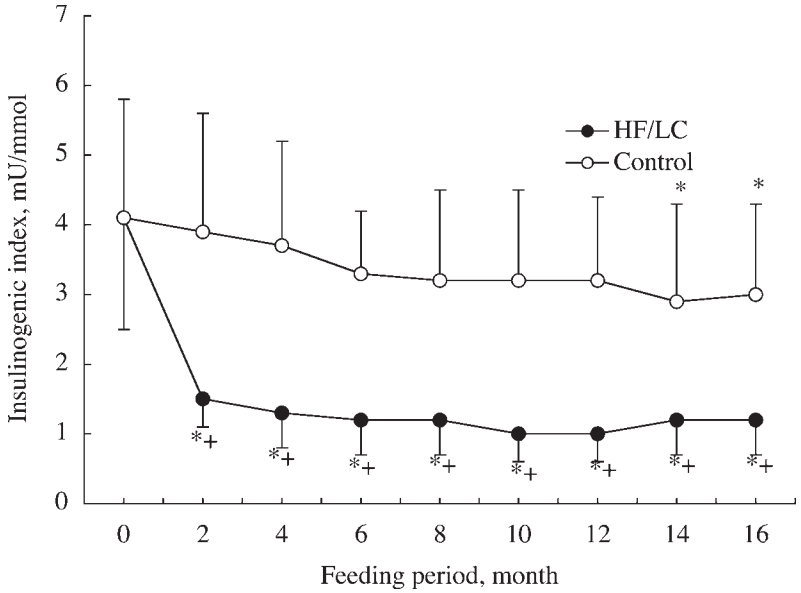

Fig. 7. Time course effects of the high-fat/low-carbohydrate diet on the insulinogenic index. Values are means ± SD. HF/LC stands for high-fat/low-carbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

2 to 12 mo of the experiment, the HOMA-IR in HF/LC rats was significantly higher than that in Control rats ($p < 0.05$). However, at 14 and 16 mo, there were no significant differences in the value between the two groups.

Fasting Plasma Lipids at Autopsy

After 16 mo of feeding, the HF/LC rats had significantly higher triglyceride (TG), total cholersterol (T-Chol), and nonesterified fatty acids (NEFA) levels than Control rats ($p < 0.05$, Table 1). The plasma high density lipoprotein–Cholesterol (HDL-Chol) in HF/LC rats was significantly lower than that in Control rats ($p < 0.05$, Table 1).

Discussion

Previous studies have suggested that a long-term HF/LC diet reduces glucose tolerance or insulin resistance in laboratory animals *(7–13)*. However, the time of dietary treatment is not adequate to induce diabetes mellitus. In present study, we observed the effect of HF/LC diet for a long period on the onset of diabetes mellitus in rats.

Although the same amount of calories were given to both groups, the body weights of the rats fed the HF/LC diet were significantly higher than those given the control diet (Fig. 1). Rebuffe-Scrive et al. *(17)* reported that a high-

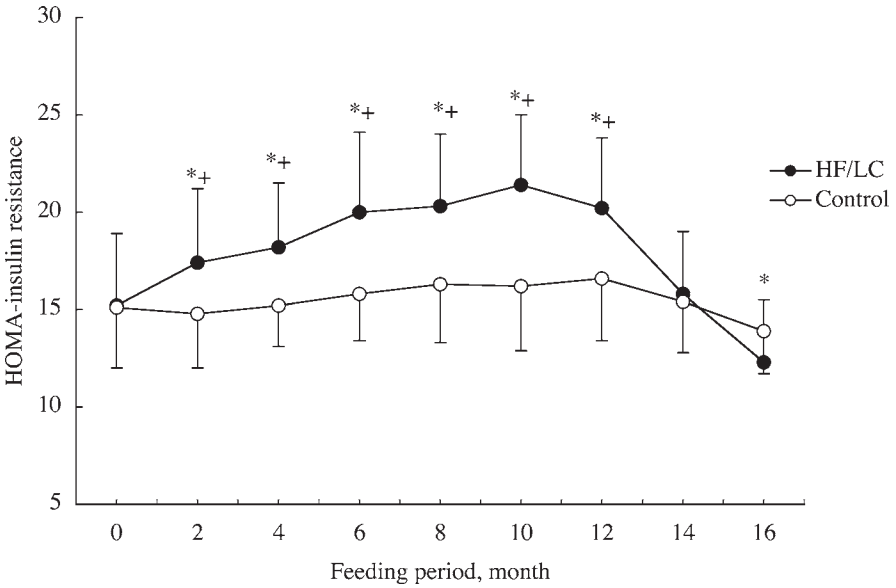

Fig. 8. Time course effects of the high-fat/low-carbohydrate diet on the HOMA-IR. Values are means ± SD. HF/LC stands for high-fat/lowcarbohydrate diet group. *Significantly different from the value at beginning (0 mo) of the same group. +Significantly different from the Control group of the same feeding period.

Table 1
Plasma Lipids at the End of Experiment (16 mo)

	TG[1] (mg/dL)	T-Chol[2] (mg/dL)	HDL-Chol[3] (mg/dL)	NEFA[4] (mmol/L)
Control	115 ± 23	102 ± 20	56 ± 9	348 ± 40
HF/LC	141 ± 29*	133 ± 25*	49 ± 8*	468 ± 59*

*Significantly different from the Control group, $p < 0.05$. [1]Triglyceride, [2]total cholersterol; [3]HDL-cholesterol; [4]non-esterified fatty acids.

fat/high-simple carbohydrate diet increased fat mass in rats, and Storlien et al. *(8)* found that high-fat feeding induced rats to accumulate more adipose tissue. In present study, the increase of fat mass in the rats fed HF/LC was presumed to be greater than those kept on the control diet. The enhanced plasma TG and NEFA may induce an increase in fat mass, and, consequently, an increase body weight in HF/LC rats *(18,19)*.

The body weights in the HF/LC group decreased sharply after 14 mo of dietary treatment (Fig. 1). The reason was presumed to be age and onset of diabetes mellitus in many rats. This suggests that feeding with a high-fat/low-carbohydrate diet for more than 1 yr is required to show detrimental effects on metabolism in rats.

We reported previously that feeding with a HF/LC diet for 3 d induces impaired glucose tolerance *(12)*. In this study, the AUC and 120-min postload plasma glucose in the HF/LC group increased significantly at the first IPGTT (2 mo). A significant rise in FPG was seen at 6 mo in the HF/LC group. HF/LC diet appeared to deteriorate the glucose tolerance prior to the increase in FPG.

A high-fat diet was demonstrated to reduce the insulin secretion from β cells in laboratory animals *(10,11)*. In the present study, administration of the HF/LC diet caused hyperinsulinemia from 2 through 12 mo as shown by the significant increase in FPI (Fig. 5), without any accompanying increase in the insulin level in response to glycemic stimulus, as is seen in the 20-min postload insulin concentration (Fig. 6). Nevertheless, both the FPI and the 20-min postload insulin level in the HF/LC rats were markedly reduced after 12 mo (Figs. 5 and 6) as compared with those in the control group. This suggests that long-term feeding with a HF/LC diet suppresses the secretion of insulin in rats.

Curry et al. *(20)* reported that the ability to secrete insulin from β cells declines in older rats. In the present experiment, the FPI in the Control rats and the 20-min postload plasma insulin in both groups decreased at the end of the experiments (Figs. 5 and 6). In relation to the decrease in body weight at approx 14 mo in both groups, this suggests that the insulin secretion is not sufficient to sustain the body weight when rats get old.

A high-fat diet has been shown to induce insulin resistance *(7–9,12,13)*. In the present study, the FPI was significantly higher after 4 mo of HF/LC diet feeding (Fig. 5), accompanying the increase in the FPG level after 6 mo (Fig. 2). It is possible that the β-cells in the pancreatic islets of HF/LC rats secreted a larger amount insulin to meet the demands of the body. HOMA-IR, an indicator of insulin resistance**,** began to increase in HF/LC rats at 2 mo (Fig. 8). This suggested that feeding with a HF/LC diet for more

than 2 mo induced insulin resistance in rats. The greater body weight in HF/LC rats (Fig. 1) may be another factor for insulin resistance. The marked decrease of HOMA-IR in HF/LC rats at 16 mo was due to the reduction in fasting plasma insulin.

Kaneko et al. *(6)* reported that HF/LC diet aggravated diabetes mellitus in spontaneously diabetic rats. In the present study, we found that 12 rats became diabetic after eating the HF/LC diet for 6 mo, while no rat became diabetic during the same period. At the end of the 16-mo diet regimen, 15 of 18 rats became diabetic in the HF/LC group, whereas only 4 of 17 rats were diabetic in the Control group. This suggests that HF/LC diet feeding for a long period induces diabetes mellitus in normal Wister rats. However, Chalkey et al. *(13)* reported that administration of HF/LC diet for 10 mo induced insulin resistance but did not develop into diabetes mellitus. In Chalkey's study, they calculated the dose of glucose in intravenous glucose tolerance test according the body surface area, which was twice our dose. The criteria for judging diabetes was not clear in Chalkey's study. Moreover, 10 mo of HF/LC diet feeding may not be long enough to cause the marked changes in metabolism in rats.

Previously, we *(12)* demonstrated that a HF/LC diet for 3 d impaired glucose tolerance by inhibiting insulin secretion of pancreatic islets and whole body insulin sensitivity through the Randle cycle, which is the activation of the glucose–fatty acids cycle. The results of the present study indicate that long-term feeding with a HF/LC diet not only inhibits secretion and sensitivity of insulin but also induces DM in rats.

Furthermore, long-term feeding with a HF/LC diet may produce adverse effects on blood plasma lipid profiles, elevating triglycerides (TG), T-Chol, NEFA levels, and reducing HDL-Chol in the plasma (Table 1).

In conclusion, the present results demonstrated that long-term HF/LC diet feeding deteriorated secretion and sensitivity of insulin, and induced diabetes mellitus in normal Wister rats.

Materials and Methods

Animals

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

Forty 8-wk-old male Wistar Imamichi rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were kept individually in stainless-steel wire-bottomed cages in an air-conditioned room (22 ± 2°C, 55 ± 10% relative humidity) with artificial lighting from 06:00 to 18:00. They were maintained on commercial powdered food (Clea CE-2, Nippon Clea, Tokyo, Japan) and water *ad libitum* for 1 wk. At 9 wk of age, the animals were randomized to either the Control or HF/LC diet. Both of these diets were semipurified powder diets. The Control diet consisted of 60% carbohydrates, 15% fat, and 25% protein in calories, while the HF/LC diet consisted of 10% carbohydrates, 25% protein, and 65% fat by calories. The HF/LC diet was prepared by replacing carbohydrate in the Control diet with an isocaloric amount of fat (Table 2). The daily calorie intake was 60 kcal/rat (pair-feeding) throughout the experiment. The food was replenished daily at 16:00. Most animals consumed their daily ration by 10:00 the next day; any food remaining at 10:00, was withdrawn at this time. The rats were kept on the two kinds of diets for 16 mo.

Table 2
Composition of the Control 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 percentage of calories in parentheses.

During the 16 mo of experimental feeding, IPGTT was performed once every 2 mo. All rats fasted for 6 h (from 10:00 to 16:00 of the same day) before IPGTT. Around 16:00 on the experimental day, fasting blood (200 μL) was collected from the tail vein into hematocrit tubes, then rats were administered 2.0 g/kg glucose (20%, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) intraperitoneally. Blood was collected from the tail vein at 20, 60, and 120 min (200, 100 and 100 μL, respectively) after glucose loading. We used 20-min post load values because the plasma insulin and glucose reached the peak at 20 min in most rats in our preliminary experiment. The increment in plasma glucose following the glucose load was expressed in terms of the area under the plasma glucose concentration time curve (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 *(15)*, was calculated using the equation (20-min plasma insulin [μU/mL])– fasting plasma

insulin [FPI])/(20-min plasma glucose [mmol/L]– fasting plasma glucose [FPG], the final unit is mU/mmol). A "homeostasis model assessment of insulin resistance" (HOMA IR) was calculated using the equation [FPI (μU/mL) × FPG (mmol/L)/22.5] *(16)*.

After 16 mo of feeding, rats were decapitated and the blood was collected after 6-h fasting (from 10:00 to 16:00). Triglyceride (TG) total cholesterol (T-Chol), high density lipoprotein–cholesterol (HDL-Chol), and nonesterified fatty acids (NEFA) were measured.

Analyses

The glucose concentration was measured with a Glucose CII-Test (Wako). 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, USA). The intra- and interassay coefficients of variation were less than 10%. The minimum detectable sensitivity was 1.2 μU/mL. Plasma TG, TChol, HDL-Chol, and NEFA level were assayed by Cholesterol-E, HDLCholesterol E, Triglyceride E, and NEFA C-Test (Wako), respectively, by using spectrophotometer (Clinical Spectrophotometer 7010 with an X-Y Autosampler, Hitachi, Tokyo, Japan).

Statistics

The data were subjected to two-way ANOVA using StatView 4.0 (Abacus Concepts, Berkeley, CA, USA). Fisher's PLSD test was used when there was a significant difference among the groups. The 0.05 level of probability was used as the criterion of significance.

Acknowledgment

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