Effect of Aging on the Development of Glucose Intolerance Induced by a Low-Carbohydrate Diet in Rats

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The effect of aging on the development of glucose intolerance between 2 kinds of diets, a low-carbohydrate/high-fat (LC/HF) diet and a control diet was investigated. A total of 20 eight-week-old S.D. rats were randomly divided into 2 groups of 10 rats each and at 10, 20, 40, 60 and 80 weeks of age, rats were switched to either control or LC/HF diet for 7 days. At 16:00 on the day following the 7th day of feeding, rats underwent an oral glucose tolerance test (OGTT). LC/HF feeding for 7 days impaired the glucose tolerance in rats. Moreover, insulin secretion decreased with age and insulin resistance increased after 60 weeks of age when rats were fed the LC/HF diet, and fasting plasma free fatty acids (FFA) concentrations increased with age. Thus, we speculated that the age-related impairment of insulin action through the glucose fatty-acid cycle might be closely associated with the onset and development of diabetes mellitus.

Key words —— aging, carbohydrate, glucose tolerance, insulin resistance, insulinogenic index, rat

INTRODUCTION

Aging is considered to be one of the important risk factors of type 2 diabetes mellitus (DM). There have been a number of reports concerning age-related development of glucose intolerance in humans,[1,2] and in an epidemiological survey, the prevalence of DM was found to increase with age in most countries of the world.[3] Moreover, the effects of aging on glucose and insulin kinetics in animals have been also reported by some investigators.[4–6] A low-carbohydrate/high-fat (LC/HF) diet is also a risk factor of DM. Kaneko *et al*.[7] reported that a dietary modification (a LC/HF diet) before a 75 g oral glucose tolerance test deteriorated glucose tolerance in humans. Moreover, rats kept on a LC/HF diet for more than 3 days before a glucose tolerance test showed impaired glucose tolerance.[8]

To our knowledge, there have been few reports on the correlations between age and diet on glucose tolerance. Reaven *et al*.[9] investigated the effect of age and diet on insulin secretion and insulin action in rats. However, the diets that they used were not isocaloric and the concentrations of nutrients were not constant. In this study, we gave test diets for 7 days to rats of various ages up to 80 weeks old, which corresponds to middle-age in humans, and we compared the effect of aging on the development of glucose intolerance between the 2 kinds of diets, a LC/HF diet and a control diet using a pair-feeding method.

MATERIALS AND METHODS

A total of 20 eight-week-old male S.D. rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were kept individually in stainless steel wire-bottomed cages in an air-conditioned room (22 ± 2°C) with artificial lighting from 6:00 to 18:00. They were maintained on commercial powdered food (Clea CE-2, Nippon Clea, Tokyo, Japan) and water ad libitum. The animals were randomly divided into 2 groups of 10 rats each and at 10, 20, 40, 60 and 80 weeks of age, switched to 2 types of semi purified powder diets, *i.e*. a control diet (with a caloric ratio of 60% carbohydrates, 15% fat and

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25% protein) and a LC/HF diet (with a caloric ratio of 10% carbohydrates, 65% fat and 25% protein) for 7 days. The diet was prepared by modifying the formulation used by Lieber and DeCarli (Table 1).[10] Ethyl linoleate was substituted for safflower oil. The two kinds of diets were adjusted to contain the same amounts of fibers, minerals and vitamins. The daily calorie intake of each diet was fixed at 80 kcal/rat throughout the experiment (pair-feeding). 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. After 7 days on the diet, all rats were returned to the same commercial powdered food. The groups of rats were randomly assigned to either the control or LC/HF diet.

At 16:00 on the day following the 7th day of feeding, rats received glucose (2.0 g/kg) solution (50%, 4.0 ml/kg) perorally (oral glucose tolerance test, OGTT). Blood from the tail vein was collected in hematocrit tubes before and 30, 60 and 120 min after glucose loading and the plasma was immediately separated and submitted for analysis of free fatty acids (FFA) at the fasting level, which was performed using a test kit (NEFA C-Test) from Wako Pure Chemicals (Osaka, Japan) with a spectrophotometer (Clinical Spectrophotometer 7010 with X-Y Autosampler, Hitachi, Tokyo, Japan). The remaining plasma was stored in ice-cold water and later analyzed for glucose (fasting plasma glucose, FPG and plasma glucose at 30, 60 and 120 min after glucose loading) and insulin (fasting plasma insulin, FPI and plasma insulin at 30 and 120 min after glucose loading). The glucose concentration was measured with a test kit (Glucose CII-Test) from Wako. The insulin concentration was determined by an Insulin ELISA Kit from Morinaga Biochemistry (Yokohama, Japan) with a microplate spectrophotometer system (SPECTRAmax 340 with SOFTmax PRO version 2.1 software, Molecular Devices, Sunnyvale, CA, U.S.A.). The increment of plasma glucose/insulin following the glucose load was expressed in terms of the areas under the plasma glucose/insulin concentration-time curve (g-AUC/i-AUC) for the time between when the fasting blood was drawn until 120-min postload blood sampling, using the trapezoidal rule. An "insulinogenic index," defined as the ratio of the change in circulating insulin to a change in the corresponding glycemic stimulus, was calculated using the equation (30 min plasma insulin – FPI)/(30 min plasma glucose – FPG).[11] This value was used as the index of insulin secretion. The hemostatic model assessment index (HOMA, the index of insulin resistance) was calculated according to Matthews *et al*.[12]

Table 1. The Composition of the Test Diets

Component[a)]	Control diet	LC/HF diet
Carbohydrate	60.0 (60.0)	13.8 (10.0)
Dextrin	30.0 (30.0)	6.9 (5.0)
Maltose	30.0 (30.0)	6.9 (5.0)
Protein	24.5 (25.0)	34.6 (25.0)
Casein Na	24.5 (24.5)	33.9 (24.5)
L-Cystin	0.3 (0.3)	0.4 (0.3)
DL-Methionine	0.2 (0.2)	0.3 (0.2)
Fat	6.7 (15)	40.0 (65.0)
Coin oil	1.3 (2.9)	8.9 (14.4)
Olive oil	4.3 (9.6)	29.6 (48.1)
Ethyl linoleate	1.1 (2.5)	1.5 (2.5)

a) g/100 g with % calories in parentheses.

The experiments were performed in accordance with the Guidelines for Animal Experiments of the Yamanashi Medical University, which are in agreement with the US National Institute of Health guidelines.

Statistics: The data were subjected to one-way analysis of variance (ANOVA) using StatView 5.0 (Abacus Concepts, Berkeley, CA, U.S.A.). Student's paired or unpaired t tests were used to determine the significance of differences among groups. A 0.05 level of probability was used as the criterion of significance. All values are given in the tables as mean ± S.D.

RESULTS

Body Weight Changes

There were no significant differences in body weights between the LC/HF and control groups at any of the experimental ages examined (10 weeks of age, LC/HF *vs*. control = 339 ± 10 g *vs*. 340 ± 7 g; 20 weeks of age, 495 ± 23 g *vs*. 494 ± 25 g; 40 weeks of age, 591 ± 27 g *vs*. 589 ± 28 g; 60 weeks of age, 607 ± 28 g *vs*. 604 ± 28 g; 80 weeks of age, 625 ± 26 g *vs*. 622 ± 21 g).

Plasma Glucose Responses after OGTT

FPG values did not significantly different between these 2 groups throughout the experimental period (Table 2). However, postload plasma glucose values (30, 60, 120 min) were significantly higher

Table 2. Plasma Glucose Responses before and after OGTT

Group	Glucose (mg/dl)				AUC (mg/dl × hr)
	before	30 min	60 min	120 min	
10 weeks of age					
Control	121 ± 10	148 ± 15	141 ± 7.9	118 ± 9.2	30.3 ± 10
LC/HF	119 ± 10	172 ± 15*	154 ± 11*	154 ± 11*	47.3 ± 10*
20 weeks of age					
Control	126 ± 11	153 ± 19	145 ± 14	122 ± 13	29.5 ± 16
LC/HF	121 ± 13	178 ± 16*	157 ± 10*	138 ± 17*	47.2 ± 8.8*
40 weeks of age					
Control	130 ± 11	155 ± 22	149 ± 17	122 ± 13	30.3 ± 18
LC/HF	127 ± 11	182 ± 21*	167 ± 12*	146 ± 19*	48.6 ± 16*
60 weeks of age					
Control	136 ± 11	162 ± 23	155 ± 17	129 ± 13	31.2 ± 19
LC/HF	136 ± 10	193 ± 23*	179 ± 14*	155 ± 20*	51.8 ± 16*
80 weeks of age					
Control	145 ± 13	172 ± 24	165 ± 19	137 ± 14	32.5 ± 21
LC/HF	146 ± 10	209 ± 23*	194 ± 14*	168 ± 20*	56.4 ± 14*

Values are mean ± S.D. for 10 rats. *Significantly different from Control ($p < 0.05$). LC/HF, low-carbohydrate/high-fat diet.

in the LC/HF group than in the control group at 10 weeks of age (Table 2). The area under the curve (AUC) value was also significantly higher when rats were kept on the LC/HF diet than when fed the control diet (Table 2). Similar results were observed at all weeks of age examined. Moreover, the peak concentrations increased with age (10 weeks of age, 172 ± 15 mg/dl *vs*. 148 ± 15 mg/dl; 20 weeks of age, 178 ± 16 mg/dl *vs*. 153 ± 19 mg/dl; 40 weeks of age, 182 ± 21 mg/dl *vs*. 155 ± 22 mg/dl; 60 weeks of age, 193 ± 23 mg/dl *vs*. 166 ± 17 mg/dl; 80 weeks of age, 209 ± 23 mg/dl *vs*. 172 ± 24 mg/dl in control *vs*. HC/LF, respectively).

Plasma Insulin Responses after OGTT

Up to 40 weeks of age, FPI and postload plasma insulin values (30 and 120 min) were not significantly different between the 2 groups (Table 3). The AUC values were also not significantly different (Table 3).

At 60 weeks of age, FPI value was significantly higher in the LC/HF group than in the control group, although the AUC value was not significantly different between the 2 groups (Table 3). Moreover, at 80 weeks of age, FPI and postload plasma insulin 120 min values were significantly higher, and postload plasma insulin 60 min values was significantly lower in the LC/HF group than in the control group, although the AUC value was not significantly different between these 2 groups (Table 3).

Insulinogenic Index and HOMA Index

In all experimental ages, insulinogenic indices in the LC/HF group were significantly lower than those in the control group (Table 4).

Until 40 weeks of age, HOMA did not significantly differ between these 2 groups (Table 4). However, HOMA indices in the LC/HF group were significantly higher than those in the control group after 60 weeks of age (Table 4).

Plasma FFA Concentrations

At all experimental ages, rats fed the LC/HF diet had significantly higher plasma FFA values than rats fed the control diet (Table 4).

DISCUSSION

There have been many reports demonstrating the relationship between aging and the development of DM. Berthelier *et al*.[4] evaluated the influence of age on insulin resistance in a genetic lean model of DM, the GK rat, using the euglycemic-hyperinsulinemic clamp technique until 18 months of age. They concluded that there was no major alteration in insulin action due to aging in this rat and that this adaptation could be related to the limited capacity of this rat to increase body weight with age, since it is recognized that body weight gain is largely responsible for the age-related impairment in peripheral insulin action in nondiabetic humans and animal models.

Table 3. Plasma Insulin Responses before and after OGTT

Group	Insulin (ng/ml)			AUC (ng/ml × hr)
	before	30 min	120 min	
10 weeks of age				
Control	1.14 ± 0.11	2.27 ± 0.13	1.33 ± 0.06	1.08 ± 0.12
LC/HF	1.20 ± 0.14	2.16 ± 0.18	1.39 ± 0.10	0.91 ± 0.28
20 weeks of age				
Control	1.18 ± 0.17	2.27 ± 0.15	1.34 ± 0.12	1.05 ± 0.22
LC/HF	1.24 ± 0.13	2.18 ± 0.24	1.43 ± 0.13	0.90 ± 0.25
40 weeks of age				
Control	1.22 ± 0.17	2.46 ± 0.34	1.53 ± 0.15	1.08 ± 0.46
LC/HF	1.33 ± 0.16	2.24 ± 0.31	1.58 ± 0.15	0.85 ± 0.36
60 weeks of age				
Control	1.12 ± 0.14	2.47 ± 0.59	1.42 ± 0.16	1.17 ± 0.63
LC/HF	1.29 ± 0.19*	2.11 ± 0.22	1.47 ± 0.16	0.80 ± 0.39
80 weeks of age				
Control	0.94 ± 0.17	2.00 ± 0.30	1.07 ± 0.21	1.02 ± 0.38
LC/HF	1.11 ± 0.15*	1.70 ± 0.27*	1.28 ± 0.15*	0.60 ± 0.26

Values are mean ± S.D. for 10 rats. *Significantly different from Control ($p < 0.05$). LC/HF, low-carbohydrate/high-fat diet.

Table 4. Insulinogenic Index (I.I.), HOMA Index, and Fasting Plasma FFA Concentration

Group	I.I. (ng/mg)	HOMA	FFA (mEq/l)
10 weeks of age			
Control	4.74 ± 2.2	6.15 ± 0.6	0.55 ± 0.11
LC/HF	2.00 ± 1.0*	6.35 ± 0.7	0.66 ± 0.10*
20 weeks of age			
Control	5.17 ± 3.9	6.68 ± 1.3	0.57 ± 0.11
LC/HF	1.80 ± 0.7*	6.64 ± 1.1	0.68 ± 0.13*
40 weeks of age			
Control	6.10 ± 3.4	7.04 ± 1.3	0.59 ± 0.12
LC/HF	1.72 ± 0.9*	7.49 ± 1.2	0.72 ± 0.13*
60 weeks of age			
Control	4.98 ± 2.6	6.82 ± 1.0	0.67 ± 0.13
LC/HF	1.54 ± 0.6*	7.72 ± 1.1*	0.82 ± 0.15*
80 weeks of age			
Control	5.26 ± 3.6	6.09 ± 1.3	0.80 ± 0.13
LC/HF	1.01 ± 0.4*	7.18 ± 0.9*	0.93 ± 0.14*

Values are mean ± S.D. for 10 rats. *Significantly different from Control ($p < 0.05$). LC/HF, low-carbohydrate/high-fat diet.

Tessari[1] reported that higher FFA kinetics reflect established changes in fat mass, and insulin sensitivity on glucose metabolism is usually normal in the aged, despite subtle impairments in insulin secretion, hepatic uptake, and onset of action. Tessari suggested that this was due to the Randle cycle;[13] an inverse relationships between fat and glucose oxidation in the elderly. Ikegami *et al.*[2] suggested that aging is associated with glucose intolerance and insulin resistance. According to Higgins *et al.*,[5] insulin responsiveness in adipose tissue of older animals may underlie the increased adiposity, which was speculated to be the causative factor in the development of age-related insulin resistance. Escriva *et al.*[6] reported that during aging in Wistar rats and before fasting plasma insulin and glucose levels became altered, specific tissues develop insulin resistance, whereas other tissues remain insulin sensitive, and suggested that fat tissue plays a qualitatively important role in eliciting insulin resistance in elderly animals. Taking into consideration the metabolic characteristics of the aged Wistar rat, we speculated that the changes reported might reflect what occurs in non-obese elderly humans, who nongenetically develop DM. In this experiment, insulin secretion (insulinogenic index) also decreased with age and insulin resistance (HOMA) was increased after 60 weeks of age when rats were fed an LC/HF diet, and fasting plasma FFA concentrations increased with age. Thus, we speculated that the age-related impairment in insulin action through the Randle cycle may be closely associated with the onset and development of DM.

Himsworth[14] reported that glucose tolerance and insulin sensitivity in healthy men were affected only by the amount of carbohydrate in the diet, but were not related to total calorie intake, amount of protein or fat, or the carbohydrate/fat ratio in the diet. To confirm this, Himsworth maintained the carbohydrate and protein amounts at constant valves of 250 g

and 80 g, respectively, while increasing the amount of fat from 100 g, 200 g to 300 g (40–67% of calories from fat) using three kinds of diets. He found that after 7 days on these diets, the dietary fat did not affect glucose tolerance, which remained unchanged as long as the carbohydrate intake remained constant.

Recently, Wang *et al*.[8] reported that glucose tolerance in rats was impaired by 3-day feeding with a low-carbohydrate diet. Similar to the results of the present study, the impairment of glucose tolerance after LC/HF diet feeding was accompanied by an increase in the FFA level in the fasting plasma, suggesting that the impairment is associated with the Randle effect, an activation of the glucose fatty-acid cycle.[13] Randle[15,16] and Randle *et al*.[17] proposed that increased FFA inhibits glucose oxidation, stimulates hepatic glucose production and inhibits the secretion of insulin by beta-cells in the pancreatic islets of Langerhans in response to glucose. Further studies are needed to clarify the mechanism underlying glucose intolerance induced by low-carbohydrate diets. In the present study, the impaired glucose tolerance induced by a LC/HF diet was further enhanced by aging. Reaven *et al*.[9] investigated the effect of age and diet on insulin secretion and insulin action in the rat. They concluded that aging leads to marked changes in both insulin secretion and insulin action. The decline in glucose-stimulated insulin secretion is an inevitable consequence of the aging process. In contrast, age-related changes in islet size, insulin response to a glucose load, and *in vivo* insulin-stimulated glucose uptake are markedly responsive to variations in the amount and kind of calories. In conclusion, our data demonstrated that the older rats were susceptible to diet (LC/HF) induced-glucose intolerance.

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