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Effect of a High-fat Diet on Diabetic Mother Rats and Their Offspring through Three Generations

RITSUKO NASU*,**, KOJI SEKI***, MISA NARA***, MASAMI MURAKAMI*** AND TOMOKO KOHAMA*

*Department of Health and Nutrition, Takasaki University of Health and Welfare, 1830-3, Nakaoorui-machi, Takasaki, Gunma 370-0033, Japan

**Department of Molecular and Cellular Pharmacology Faculty of Medicine, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan

***Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan

Abstract. Pregnant diabetic Wistar rats were fed a high-fat diet starting at the first gestational day. The effect of the high-fat diet on the growth of the female, her offspring, and the offspring's offspring was studied. Pregnant rats (first generation) were divided into the Diabetic streptozotocin-induced group and the control group. Diabetic streptozotocin-induced rats and control rats were fed either a control diet (5% fat in diet) or high-fat diet (32% fat in diet), and observed up to the third generation. In each generation, after weaning, the pups were fed the respective diet. The fat content was mainly animal lard. Diabetic rats fed the high-fat diet were infertile, and the pregnant first-generation and diabetic rats fed the control diet had a stillbirth rate of 27.5 ± 22.0% (mean ± SE). In the first generation, the diabetic rats fed the control diet had a significantly lower body weight increase during the pregnancy than the control rats fed the control diet. The second-generation diabetic rats fed the control diet had a high blood glucose level at birth, and their triglyceride level was higher than that in the other two groups. The third-generation diabetic rats fed the control diet had a triglyceride level higher than that of control rats. Delivery was most difficult in diabetic rats fed the high-fat diet. Pups of diabetic rats fed the control diet had growth retardation and increased blood glucose levels. We conclude that when the mother rat had diabetes, the next generation was also affected.

Key words: Diabetic rat, Pregnancy, High-fat diet, Offspring

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IT has been known that the newborns of diabetic mothers are frequently macrosomic in humans [1]. Hypernutrition during pregnancy causes hyperinsulinemia in the fetus, making the newborn macrosomic. Risk factors for giving birth to macrosomic newborns include maternal gestational diabetes, obesity, and excessive body weight. The risk factors can be removed by nutrition management [2, 3]. It has been reported that in animals diabetes in a pregnant mother affects her own lipid metabolism as well as that of her offspring [4, 5]. When a high-fat diet was given to pregnant female rats, pancreatic cell development in the fetuses deteriorated and the newborns were hyperglycemic [6]. Even in women with normal pregnancies, unbalanced nutrition during the pregnancy affects the growth of her offspring after birth. The newborns of mothers with a nutritional disorder have low body weight associated with insulin secretion insufficiency. Twelve-week-old offspring of diabetic female rats had impaired glucose tolerance [7].

In this study, pregnant diabetic rats were fed a high-fat diet during pregnancy and the suckling period, and the influence of diet on the mother and offspring and the subsequent growth of the mother and offspring were studied.

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Correspondence to: Ritsuko NASU, Department of Health and Nutrition, Takasaki University of Health and Welfare, 1830-3, Nakaoorui-machi, Takasaki, Gunma 370-0033, Japan

Material and Methods

This study was approved by the Animal Committee of Takasaki University of Health and Welfare. 12-week-old female Wistar rats were mated with male Wistar rats. Pregnant 12-week-old Wistar rats on the first gestational day were divided into the Diabetic (D) group and control (C) group. Two types of diets were administered to the Diabetic and control groups starting on the first gestational day: a control diet (N; 5% fat, Nippon Clea Company, Tokyo, Japan) or a high-fat diet (F; 32% fat). The fat consisted of mainly animal lard (Table 1). Diets and water were provided *ad libitum*. The diet was started on the first gestational day before streptozotocin (STZ) administration. On the second day of gestation, STZ (50 mg/kg, STZ) in 0.2 ml of saline was injected in the tail vein to induce diabetes in the Diabetic groups and saline alone was injected in the pregnant rats in the control groups. Streptozotocin was only administered to the first generation of pregnant rats. The four groups, C-N, C-F, D-N, and D-F, were continuously bred up to the third generation. Each generation after weaning of pups was fed the respective diet. All adult female rats were bred with normal Wistar male rats. These pregnant Wistar rats were considered to be the first generation, and their offspring was considered to be the second generation. Rats were housed individually in woodchip-bedded plastic cages at a room temperature of 25°C and humidity of 60 ± 5% with a 12-hour light-dark cycle. Handling of the animals conformed with the Guidelines for the Care and Use of Experimental Animals (Prime Minister's office notification, No. 6). In the mother rats, body weight at delivery, blood glucose level, serum triglyceride level and organ weights were measured. Blood samples were collected from the tip of the tail of pregnant rats at 1, 7, 14, and 21 days of gestation and the postprandial blood glucose level was measured. Triglyceride level was measured after overnight fasting. At delivery, body weight was measured in all newborns. Half of the newborn rats were sacrificed by decapitation. The remaining female rats of the second generation at 14 weeks of age were mated with normal Wistar male rats (Clea, Tokyo). After mating, the first day of gestation was estimated by the presence of spermatozoids in vaginal smears. Similar to the first generation, those rats were bred.

Table 1. Composition of the diets (g/100 g of diet)

	Control	High fat
Moisture (%)	8.6	6.2
Crude protein (%)	24.9	25.5
Crude fat (%)	4.6	32
Crude fiber (%)	3.7	2.9
Crude ash (%)	6.7	4
NFE (%)	51.4	29.4
Calorie (kcal)	346.8	507.6
Fat kcal (%)	11.9	56.7

Postprandial blood samples were obtained from newborn female rats on days 0, 7, 14 and 21 of life, and the blood glucose level was determined. In the second-generation rats, body weight at delivery, blood glucose level, serum triglyceride level, and organ weights were measured. In the third-generation rats, body weight at delivery, blood glucose level, serum triglyceride and blood fluidity levels were measured. After the pups were weaned, all adult female rats were sacrificed at 17 weeks of age. The rats were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight). Whole blood was drawn from the heart into tubes. Serum was obtained by centrifugation at 3,000 × g at 4°C for 10 min. The serum samples were kept frozen at –80°C until determination of the serum triglyceride level. The serum triglyceride level was measured by enzymatic procedures (Wako, Tokyo). Weights of the liver, kidneys, spleen, and heart were measured at autopsy.

In the newborn rats that were sacrificed immediately after delivery, blood was collected by decapitation immediately after birth. Serum was kept frozen at –80°C until analysis for determination of the triglyceride level.

The delivery rate was defined as the number of liveborn births/(number of liveborn births + number of stillbirths) per mouse. The stillbirth rate was defined as the number of stillbirths/(number of liveborn births + number of stillbirths) per mouse. Data are presented as mean ± standard error (SE). Differences among the groups were analysed for significance by ANOVA, followed by Scheffe's multiple range test. Significance was set at $P<0.05$.

Results

1) Stillbirth rate

The stillbirth rate of pregnant female rats in each

generation in each group is shown in Table 2. In the first generation, there were no newborns born to the diabetic rats fed the high fat diet.

In the second generation, the mean stillbirth rate was 27.5% ± 22.0% (SE) among the first-generation diabetic rats fed the control diet (D-N). The stillbirth rate in the diabetic rats fed the control diet was significantly higher than that in the control rats fed the control diet (27.5 ± 22.0% vs. 0%, $p<0.05$).

In the third generation, the delivery rate in the Control rats fed the control diet, Control rats fed the high-fat diet, or Diabetic rats fed the control diet was 100%. Stillbirths occurred in the C-N, C-F and D-N groups, although the stillbirth rates were low. There were no deaths on the day of delivery in the third generation in any of the groups.

2) Blood glucose level

Changes in the blood glucose level over time in the first-generation pregnant rats and in the second-generation and third-generation newborns are shown in Fig. 1A, Fig. 1B and Fig. 1C. Among the first-generation pregnant female rats, during the course of the pregnancy, the blood glucose level in the D-N and D-F groups was significantly higher than that in the C-N and C-F groups on gestational day 21 ($p<0.01$ each) (Fig. 1A). Among the second-generation pregnant females, blood glucose levels in the Control groups were within the normal limits and decreased during the course of the

Table 2. Litter size of Diabetic and Control rats fed the high-fat diet or control diet

Group	No. of litters in group			No. of rats/litter		No. of stillbirth rate/litter	
Generation	1	2	3	2	3	2	3
C-N	3	4	3	9.3 ± 5.1	13.8 ± 1.5	0**	3.5 ± 3.5
C-F	4	8	4	11.3 ± 1.5	8.0 ± 9.9	0	3.3 ± 3.3
D-N	4	4	3	13.5 ± 0.7	13 ± 1.1	27.5 ± 22.0**	1.9 ± 3.3
D-F	4	—	—	—	—	—	—

C-N: control rats that were fed the control diet
C-F: control rats that were fed the high-fat diet
D-N: diabetic rats that were fed the control diet
D-F: diabetic rats that were fed the high-fat diet
The results are presented as mean ± S.E.. Significantly different from C-C group; **$p<0.01$.

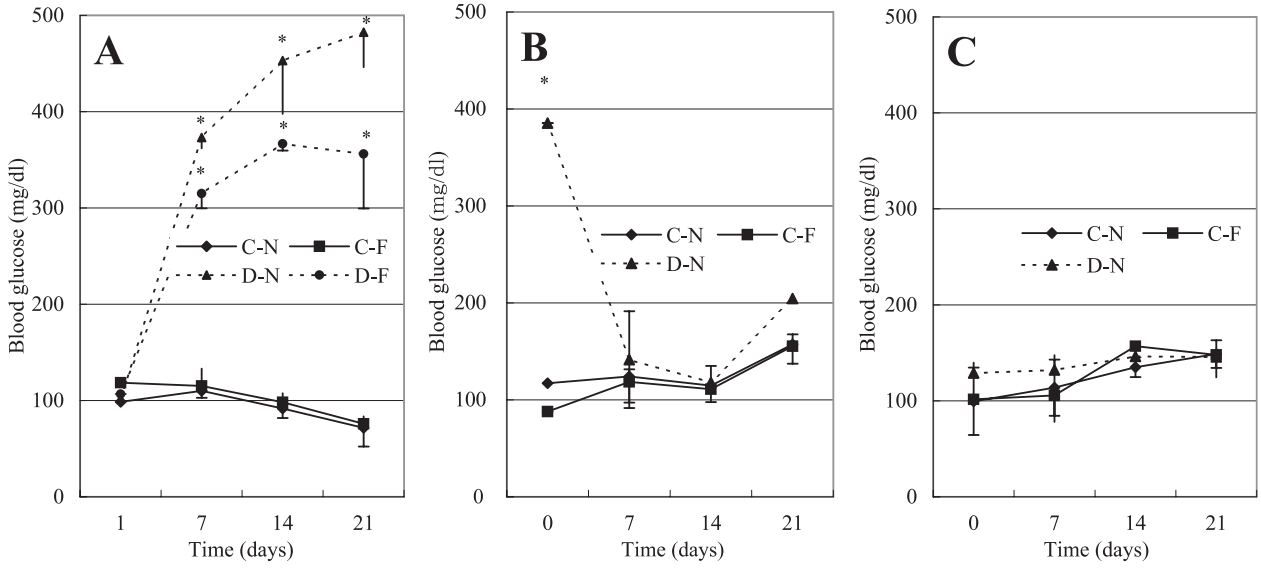

Fig. 1. Change in the blood glucose levels in (A) pregnant rats of the first generation, and newborn rats of the (B) second and (C) third generations. *$p<0.05$, significantly different from the C-N group. In (A), day 1 corresponds to the day in which the high-fat diet or control diet was started in pregnant female rats. In (B) and (C), day 0 corresponds to the day of birth.

Table 3. Change in body weight from birth to weaning (4 weeks of age) (g) during the pregnancy, body weight of newborns, and change in body weight from birth to weaning

	Generation	Group					
		n	C-N	n	C-F	n	D-N
Average body weight immediately after delivery (g)	1	3	240.0 ± 34.1*	4	251.0 ± 15.9	4	199.0 ± 17.3*
	2	4	291.2 ± 16.7	8	305.5 ± 9.4	4	246.3 ± 9.4
Change of body weight during pregnancy (g)*[1]	1	3	27.7 ± 16.8*	4	28.0 ± 4.5	4	–24.7 ± 6.9*
	2	4	31.3 ± 11.6	8	52.0 ± 20.0	4	47.0 ± 8.9
Average body weight at the time of weaning (g)	2	12	53.1 ± 4.5*	15	60.4 ± 12.2	9	17.7 ± 0.5*
	3	20	53.2 ± 2.2	15	57.2	20	50.5 ± 1.9
Change of body weight at the time of weaning (g)*[2]	2	12	48.0 ± 4.3*	15	48.3 ± 1.7	9	12.7 ± 1.5*
	3	20	46.0 ± 3.9	15	52.4 ± 1.5	20	45.0 ± 0.5
Average body weight of newborns (g)	2	27	5.3 ± 0.8	44	5.0 ± 0.6	42	5.1 ± 0.4
	3	42	5.4 ± 0.4*	64	5.0 ± 0.4*	43	5.5 ± 0.5

Values are expressed as mean ± S.D. *: $p<0.05$

*[1] (Body weight immediately after delivery) – (Initial body weight on pregnancy).

*[2] (Body weight at the time of weaning) – (Body weight at birth).

pregnancy. Among the second-generation newborns, blood glucose levels in the C-N and C-F group were normal. The blood glucose level in the D-N group was significantly higher than that in the two control groups on the first day of life ($p<0.01$, $p<0.01$) and tended to be higher than that in the other two groups during the course of growth (Fig. 1B). At the time of weaning (day 21), the blood glucose level of the D-N group was 205 ± 57 mg/dl and was significantly higher than that in the C-N and C-F groups ($p<0.05$, $p<0.05$). However, as the animals grew, the difference in blood glucose level between the D-N group and the two control groups became less and eventually disappeared. Among the third-generation newborns, there was a tendency for the blood glucose level to be lower in the D-N group than in the two control groups at birth (perinatal hypoglycemia) (Fig. 1C). However, the blood glucose level recovered as they grew.

3) Body weight

Change in body weight during the pregnancy, average body weight of newborns, and change in body weight at weaning (4 weeks of age) are shown in Table 3. In the first-generation pregnant females, the degree of the increase in body weight during the pregnancy was significantly lower in the diabetic rats fed the control diet than in the control rats fed the control diet.

In the third generation, the newborns' average body weight was significantly lower in the C-F group than in the C-N group. Also, in later growth, the C-F group tended to have a lower body weight than the C-N group. The magnitude of the increase in body weight from birth to weaning was significantly lower in the third-generation D-N group than in the C-N group (12.7 g vs. 48.0 g, $p<0.05$). The magnitude of the increase in body weight from birth to weaning in the third-generation pups was significantly smaller in the C-F group than the C-N group. The magnitude of the increase in body weight from birth to weaning tended to be smaller in the C-F group than in the other two groups.

4) Organ weight

In the adult female rats, the weights of organs obtained after sacrifice at 16 weeks of age are shown in Table 4. The weight of the pancreas in the D-N group was significantly lower than that in the C-N group due to STZ administration. The weight of the heart was significantly lower in the D-N group than in the C-N group, suggesting that this is one of the factors leading to the low delivery rate. In the second generation, organ weights were not significantly different between the C-N group and D-N group. Autopsy findings showed visceral fat accumulation in the C-F group but not in the D-N and C-N groups.

Table 4. Weights of various organs in adult female rats

	Generation	Pancreas	Kidney	Liver	Heart
C-N	1	0.55 ± 0.14	2.12 ± 0.31	7.89 ± 1.62	1.06 ± 0.02
	2	0.48 ± 0.06	1.88 ± 0.28	7.65 ± 1.23	1.22 ± 0.09
C-F	1	0.59 ± 0.07	2.09 ± 0.31	8.63 ± 0.73	1.01 ± 0.11
	2	0.29 ± 0.1	1.97 ± 0.19	7.89 ± 1.01	1.19 ± 0.12
D-N	1	0.42 ± 0.24*	2.20 ± 0.22	8.29 ± 1.1	0.78 ± 0.10**
	2	0.37 ± 0.1	1.96 ± 0.20	8.12 ± 1.12	1.17 ± 0.15
D-F	1	0.50 ± 0.23	2.52 ± 0.22	8.57 ± 0.05	1.13 ± 0.14+

Values are expressed as mean ± S.D. Significantly different from C-N group; *p<0.05, **p<0.01. Significantly different from D-N group; +: p<0.05

Table 5. Serum triglyceride levels in adult female rats

Generation	1		2		3	
	n	TG (mg/dl)	n	TG (mg/dl)	n	TG (mg/dl)
C-N	3	57.5 ± 16.2	4	43 ± 7.1	3	10.7 ± 3.5
C-F	4	93 ± 148.1	8	32.5 ± 4.9	4	4.5 ± 0.7
D-N	4	33.5 ± 14.8	4	16 ± 5.7	3	17.6 ± 8.3
D-F	3	275.7 ± 150.0	—	—	—	—

Values are expressed as mean ± S.D.

5) Serum triglyceride levels

The serum triglyceride levels in each generation are shown in Table 5. Among the first-generation adult females, the D-F group had a significantly higher triglyceride level than the D-N group. Among the second-generation adult females, there was no significant difference between the C-N group and D-N group. Among the third-generation newborns of the second-generation adult females, on the first day of life the serum triglyceride level was significantly higher in the D-N group than in the C-N and C-F groups, and it tended to be higher in the D-N group than in the two control groups thereafter.

6) Blood fluidity

Blood fluidity of the three generations was measured by Micro Channel Array Flow ANalyzer (MC-FAN). The fluidity rate tended to be slower in the D-N group (39.3 ± 3.5 s) than in the C-N group (37.6 s).

Discussion

When pregnant diabetic rats were fed a high-fat diet, they could not deliver pups due to poor blood glucose control and worsening of maternal nutrition. Detrimental effects were observed from long-term feeding of a high-fat diet. In addition, this study revealed that once fetuses were exposed to hyperglycemia, fetal growth and later growth were affected. The weight levels of the D-N group pups in this study decreased (Table 3), which was associated with higher blood glucose levels (Fig. 1B). This is in accordance with the report of Yamada *et al*. [8] that diabetes increased energy intake but not energy gain in animals, and that the body weight of offspring of diabetic rats was lower than that of offspring of normal rats.

The reason that pups were not born to diabetic rats fed the high-fat diet may be attributed to the percentage of fat energy in the diet, which was high at 56.7%, and the fact that the blood glucose level was very high at a mean level of 346 ± 39 (SE) mg/dl on day 4 of life. Miyamoto [6] reported that when a large amount of fat in the diet was given to normal rats, they showed insulin resistance and marked hyperinsulinemia. Also, the offspring of such mothers were reported to have

high body weight at birth, hyperinsulinemia, and a reduced number of pancreatic β cells [9]. As for the fat energy ratio, Jean *et al.* [10] reported that when Wistar rats were weaned and were then allowed to choose from three types of diet, *i.e.*, a high sugar diet, high protein diet, and high fat diet, the fat energy ratio was 37 ± 7%. Konomi *et al.* [11] allowed infant rats to choose from a low-fat diet (energy ratio 9.1%) and a high-fat diet (energy ratio 43.2%), and more than 80% of the rats chose the high-fat diet; this result suggested that the rats had a high preference for the high-fat diet regardless of various influences before weaning. Although it was suggested that the rats had a high preference for the high-fat diet, the physical condition of these rats worsened compared with rats that were given the control diet, and the body weight increase of rats fed the high-fat diet was small, probably because the percentage of fat in the high-fat diet was too high in our study. Concerning adult rats, Cerf *et al*. [12] reported that they had a high preference for a high-fat diet and that their over-eating caused the increase in body weight. We confirmed the tendency of an increase in body weight with control rats (Table 3). However, we failed to detect such an increase in diabetic rats; their body weight did not increase when fed the high-fat diet (Table 3) and their physical condition worsened compared with those given the control diet. We speculate that an *ad libitum* way of feeding is responsible for the failure. Cerf *et al*. [12] also noted that blood glucose increases are associated with body weight increases and implied that decreases in body weight may decrease blood glucose levels. Our data showing a decrease in blood glucose levels in diabetic rats fed a high-fat diet (Fig. 1A) is compatible with findings in their report.

As for the blood glucose level, the second-generation infant rats of the D-N group initially had a high blood glucose level but it gradually decreased to the normal range. Miyamoto [6] reported that a change in blood glucose level was observed between birth and five weeks of age in newborns of mother rats fed a high-fat diet, while the blood glucose level of the control group was within the normal range. However, the diabetic group had hyperglycemia at birth and the blood glucose level later decreased to the normal range; similar results were obtained in the present study.

Besides blood glucose and insulin levels, there are many factors that affect the growth of children. It is known that amino acids, lipids, and ketones are also transported via maternal-infant transmission in a concentration gradient-dependent manner, producing a variety of effects on the fetus depending on the time and duration of the exposure. It has been reported that especially in the latter half of a pregnancy, fetal adipose cells and pancreatic β cells are influenced to some extent by an excess supply of those nutrients, which could lead to obesity and impaired glucose tolerance during childhood and adolescence [12, 13].

STZ-induced diabetic rats that were fed a high-fat diet could not have pups. Mildly diabetic rats may deliver pups that are macrosomic. Therefore, in future studies we will examine the pups of rats that are fed foods having various fat content. Finally, it must be noted that the β cells of the pregnant rats were destroyed by STZ while leaving the genetic background unaffected. The diabetic nature of the second and third generations provides an excellent experimental model for epigenetic studies. We are interested in changes in signal transduction molecules of organs of second- and third-generation rats.

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