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## Physiological Vulnerability to Diet Induced Obesity in Inbred Alloxan-Induced Diabetes-Susceptible Mice

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**Abstract:** The effects of high fat diet on obesity and susceptibility to diabetics in Alloxan-induced diabetes-susceptible mice were studied. A total of 40 mice were divided equally (n = 10 per group) into four groups: (i) male high fat (40%) diet group (ii) female high fat (40% fat) diet group (iii) male control (7% fat) diet group and (iv) female control (7% fat) diet group. The body weight and blood glucose level of high fat male diet group were significantly ( $p < 0.01$ ;  $p < 0.001$ ) higher than those of other groups at 5 to 20 weeks of age. The high fat male and female mice produced significantly higher ( $p < 0.05$ ;  $p < 0.001$ ) perinephrium fat and mesenteric fat than those for corresponding control groups. However, the high fat female mice produced significantly ( $p < 0.001$ ) higher epididymal and high fat male mice produced significantly ( $p < 0.001$ ) lower epididymal fat than those for corresponding control groups. The high fat female mice produced significantly ( $p < 0.01$ ) higher retroperitoneal fat than that for control female group. The percentages of pancreas and genital organ tended to decrease and percentage of liver tended to increase for high fat male mice and percentage of pancreas and liver tended to decrease in high fat female mice than those for corresponding control group. However, the difference between two dietary groups in female mice for genital organ was insignificant. Only the male group fed with high fat diet developed diabetes (rate of incidence of glycosuria) with the earliest onset in strains occurred at the age of 10 weeks, followed by increasing to 100% disease at the age of 18 weeks and the phenomenon had been kept till the 20 weeks of age. Results of this study have shown that high fat diet male mice developed diabetics and dysfunction of glucose metabolism.

**Key words:** Obesity, diabetes, high fat diet, visceral fat, mice

### INTRODUCTION

Mouse models have been employed in the study of obesity for nearly a century and have been proved valuable in elucidating the effects and causes of obesity and related phenotypes (Ravussin and Bouchard, 2000). Increased dietary fat content has been shown to produce diabetes and obesity in various strains of mice and in rats. Obesity, which is easily induced by western diet, usually results in type II diabetes (Thomas *et al.*, 2003). As an environmental factor, dietary fat is a key component that influences the metabolic pathways involved in the development of diabetes (Misato *et al.*, 2004). Several current theories exist to explain the role of fat in the development of obesity (Bray, 1989). Fat has also received recent attention as the possible stimulus for the onset of type II diabetes in susceptible individuals (McGarry, 1992). It has been reported that some inbred strains of mouse differentially developed obesity and type II diabetes in response to feeding with a high-fat diet (Misato *et al.*, 2004).

Alloxan-induced diabetes is known to be a representative form of experimental diabetes and it is induced through the destruction of  $\beta$ -cell in Langerhan's islands by alloxan (Ino *et al.*, 1991). In other experiment, Hachell *et al.* (1986) noted that the incidence of Alloxan-induced diabetes varies according to the method of administration, the environmental factors such as nutritional conditions and the species and strains of the animals used. However, there has been no report on investigation of effects of nutritional condition on Alloxan-induced diabetes-susceptible mice. In this study, the body weight, blood glucose level, visceral fat and percentage of incidence of glycosuria were examined to investigate whether or not high fat diet can induce obesity and diabetes in Alloxan-induced diabetes-susceptible male and female mice. The current study also investigates the effects of high fat diet on body weight blood glucose level and also distribution of fat deposition on different tissues including reproductive tissues.

**MATERIALS AND METHODS**

**Treatment of animals:** The Alloxan-induced diabetes-Susceptible (ALS) inbred strain from a foundation stock derived from Crj: CD-1 (ICR) mice were established after more than 20 generations of full-sib mating and by simultaneous selective breeding for developing and not developing diabetes after Alloxan administration (45 mg kg<sup>-1</sup> in males, 47 mg kg<sup>-1</sup> in females) (Ino *et al.*, 1991). They were managed at the Department of Animal Resources, Laboratory of Advanced Science Research Center of Okayama University under pathogen free conditions with a standard 12 h light/dark cycle, temperature (24±0.5°C), relative humidity (55±5%) and ventilation (10-12 times h<sup>-1</sup>). The mice were housed in cages (30×20×13 cm) after a 1 week of adaptation period to the cage. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. Male and female Alloxan-induced diabetes-susceptible inbred mice were each randomly equally divided (n = 10 per group) into four groups, which showed the uniformity in response to each treatment. The groups were: (i) male high fat diet group (ii) female high fat diet group (iii) male control diet group and (iv) female control diet group. During the study period (from 4 to 20 weeks) the high fat male, control male, high fat female and control female mice were consumed 379.82, 397.95, 362.81 and 378.77 g feed/mice, respectively.

**Experimental diet:** The high fat mice groups were fed with high fat diet containing 40% (Lard 33.0%, Soybean-oil 7.0%) fat, while the control groups contained 7% (Soybean-oil 7.0%) fat diet. The feeding trail was of 4 to 20 weeks of age. The composition of the diets is listed in Table 1.

**Body weight, blood glucose level, visceral fat and percentage of glycosuria:** The body weight (g) and blood glucose level (mg dL<sup>-1</sup>) of each animal were measured weekly upto 20 weeks of age. From each group, 6 mice

Table 1: Composition of experimental diet

Ingredient	High fat	Control
Corn starch	6.74	39.74
A-corn starch	13.2	13.2
Casein	20	20
Sucrose	10	10
Soybean oil	7	7
Lard	33	-
Cellulose	5	5
Mineral	3.5	3.5
Vitamin mixture	1	1
L-cystine	0.3	0.3
Choline bitartrate	0.25	0.25
3-Butyl hydroxide	0.0104	0.0104
Total	100	100

were sacrificed randomly at 20 weeks of age and weight of pancreas, liver and visceral fats such as epididymal fat (EPI), perinephrium fat (PER), retroperitoneal fat (PR) and mesenteric fat (MES) were measured. The visceral fats from each mouse were dissected according to defined anatomic landmarks. Percentage of incidence of glycosuria was measured in Diastixo above +(0.25 g dL<sup>-1</sup>) be judged. Blood glucose levels were measured for appeared rate glycosuria.

**Statistical analysis:** The mean values for each parameter were calculated and the statistical comparisons were made using two-tailed student t-test.

**RESULTS**

Figure 1 shows the body weights of mice for different dietary groups from 4 to 20 weeks of age. The body weights of animals of both sexes on high fat group were heavier than animals on corresponding control group from 6-weeks onward. The body weight of male mice was significantly (p<0.05) heavier and female mice was highly significantly (p<0.001) heavier over control group at 5 weeks of age. However, the body weight of male mice was highly significantly (p<0.001) and of female mice were significantly (p<0.01) heavier over control group from 6th weeks onward. It was observed that the differences in body weights between high fat and control groups was 15.3 g for male mice and 17.4 g for female mice at 20 weeks of age.

The percent of different kinds of visceral fats at 20 weeks of age are shown in Table 2. The high fat male and female mice produced significantly higher (p<0.05, 0.001) PER and MES than those for corresponding control groups. The high fat female mice produced significantly (p<0.001) higher EPI than that for control female, while the high fat male mice produced significantly (p<0.001) lower

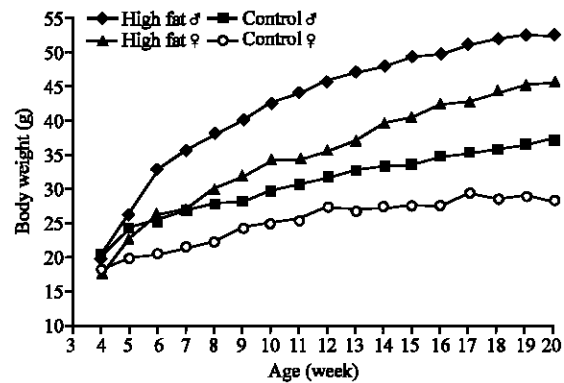


Fig. 1: Body weight of male and female mice fed with high fat and control diet from 4 to 20 weeks of age

Table 2: Visceral fat pads in male and female mice in high fat and control diets at 20 weeks of age (%)

		Mean±SEM (n = 6)				
Sex	Treatment	EPI	PER	RP	MES	Total visceral fat
Male	Control	5.21±0.21***a	0.54±0.06***b	0.75±0.06	1.13±0.08***b	7.63±0.40
	High fat	3.63±0.03***b	1.46±0.08***a	0.61±0.04	2.68±0.11***a	8.38±0.22
Female	Control	2.69±0.18***b	0.76±0.07 <sup>ab</sup>	0.34±0.03***b	0.87±0.07***b	4.65±0.33***b
	High fat	7.99±0.62***b	1.82±0.31 <sup>a</sup>	0.65±0.07***a	3.10±0.41***a	13.56±0.99***a

EPI: Epididymal fat; PER: Perinephrium fat; RP: Retroperitoneal fat; MES: Mesenteric fat; Means with different superscript between high fat and control diet within the same parameter differ significantly (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001)

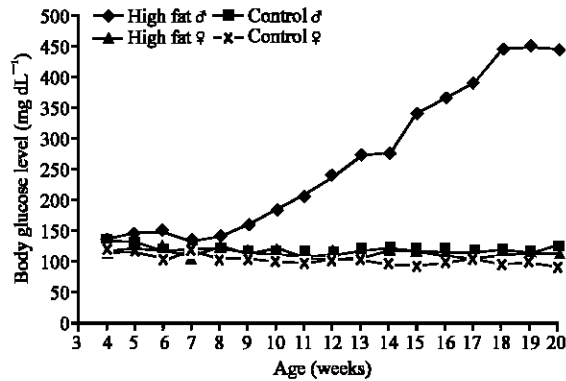


Fig. 2: Changes in blood glucose levels of male and female mice fed with high fat and control diet from 4 to 20 weeks of age

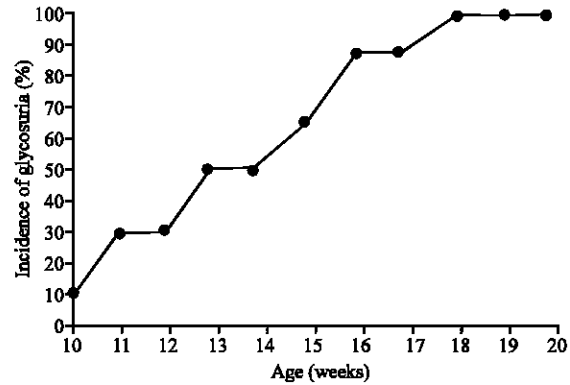


Fig. 3: Rate of incidence of glycosuria in male mice fed with high fat and control diet from 10 to 20 weeks of age

Table 3: Comparison between high fat and control diet for weight of pancreas, liver and genital organ (%) in male and female mice

		Mean±SEM (n = 6)		
Sex	Treatment	Pancreas	Liver	Genital organ
Male	Control	0.86±0.02 <sup>a</sup>	3.95±0.17***b	0.45±0.03 <sup>a</sup>
	High fat	0.73±0.02 <sup>b</sup>	6.30±0.31***a	0.31±0.02 <sup>b</sup>
Female	Control	1.00±0.05***a	4.39±0.15	0.13±0.01
	High fat	0.73±0.04***b	4.05±0.11	0.10±0.01

Means with different superscript between high fat and control diet within the same parameter differ significantly (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001)

EPI than that for control male. The RP was significantly (p<0.01) higher for high fat female than that for control female, however, the difference between two dietary groups for RP of male mice was insignificant. The percentage of total visceral-fat of high and control fat diet for female mice were 13.56 and 4.65%, respectively and the difference was statistically highly significant (p<0.001).

The comparison between high fat and control diet group mice for percentages of pancreas, liver and genital organ at 20 weeks of age are shown in Table 3. The dissect data showed that the percentages of pancreas and genital organ tended to decrease (p<0.05) and percentage of liver tended to increase (p<0.001) for high fat male mice and percentage of pancreas tended to decrease (p<0.01) in high fat female mice than those for corresponding control group. However, the difference for liver and genital organ of female mice between two dietary groups was insignificant.

Figure 2 shows the levels of blood glucose of high fat and control diet groups in male and female mice. Blood glucose levels were increased for only high fat male mice as age progressed and they produced significantly (p<0.05; p<0.01; p<0.001) higher glucose level than all the other groups, except at 4 and 7 weeks of age, which were insignificant. The high fat group female mice were also produced significantly higher (p<0.05; p<0.01) glucose level than control group at 6, 8, 10, 14, 15, 16, 18, 19 and 20 weeks of age.

Results of the present study showed that only high fat male mice developed diabetes from 10 to 20 weeks of age. The other dietary groups of mice did not show any incidence of glycosuria from 4 to 20 weeks of age. The changes of rate of incidence of glycosuria in male mice are shown in Fig. 3. The incidence of glycosuria in male mice increased sharply after showing the crisis. The first crisis appeared at the age of 10 weeks and rate of crisis was 50% at the age of 13 weeks and 100% at the age of 18 weeks still 20 weeks.

### DISCUSSION

Alloxan-induced diabetes-susceptible strain mice have been previously demonstrated to be an excellent model system for the study of complex polygenic traits related to body weight, blood glucose and obesity

(Kobayashi *et al.*, 2004). The present study establishes baseline phenotypic measurements for a variety of obesity-related traits on high fat and control diets for this strain. Results of the present study showed that animals fed the high fat diet had significantly increased body weight and blood glucose levels compared with animals fed the control diet in both the sexes.

In the present study, the total visceral fat was significantly higher for high fat diet than that for control diet in female mice, although the difference between diet groups in male mice was insignificant. These results were in agreement with the finding by Taylor *et al.* (1999), who noted that fat distribution in mice differs by strain, sex and diet. They also reported both male and female diabetes prone mice were obese and, in contrast, B6 females were slightly leaner than males. Body fat distribution and percentage of body fat have both been shown to be influenced by meal feeding, high fat diets and gender (Leibel *et al.*, 1989). Results of other studies show that distribution of fat deposition differs among different tissues in visceral fat (George *et al.*, 1990). At the present study males stored proportionally less fat in the reproductive fat pad on a high-fat diet and store proportionally more fat in the renal fat pad in response to a high-fat diet. This result was consistent with the published result by Thomas *et al.* (2003). In the present study mice that on a high fat diet gained more total visceral fat and specifically increased their MES fat depot in comparison to control group. These results might be showed that the increased MES fat deposition observed in this animal model is associated with visceral fat. Different studies have shown that adipose tissue is not only a depot for fuel storage in the form of triglyceride, but also secretes a variety of biologically active molecules. The visceral fat has a high density of glucocorticoid receptor (Rebuffe *et al.*, 1985), a high lipoprotein lipase activity and triglyceride-fatty acid uptake (Marin *et al.*, 1990), a high sensitivity to the main lipolytic hormone, norepinephrine (Rebuffe *et al.*, 1985) and a decreased sensitivity to the antilipolytic effect of insulin (Bolinder *et al.*, 1983). These later characteristics would result in a high release of free fatty acids from visceral adipose tissues into the portal circulation. This, in turn, might be a potential explanation for the increased risk factors associated with an enlargement of the visceral fat mass (Bjorntorp, 1989).

The weights of liver and genital organ in male mice responded dramatically to diet, while in the female mice it was not different between diet groups. These results are partially supported by Baron *et al.* (1988), who reported that liver and skeletal muscles are the two most important insulin-responsive organs in the body, which respond to diet. The weight of pancreas on high fat group male mice was decreased in both the sexes. It might be viewed as a failure of the pancreatic  $\beta$ -cell to compensate for

peripheral insulin resistance with enhanced insulin secretion. After a long-term, high-fat diet, the  $\beta$ -cell proliferation markedly decreased and islet cellular senescence was observed (Sone and Kagawa, 2005). In type II diabetes, the function of reduced  $\beta$ -cell is observed and  $\beta$ -cell mass is decreased by as much as 50% (Butler *et al.*, 2003). Previous studies in rats showed that 90% of pancreas must be removed in order to increase the glucose level (Hosokawa *et al.*, 1996).

Kobayashi *et al.* (2004) reported that increases in fat deposition have been shown to have tissue-specific relationships with activity of adipose tissue lipoprotein lipase activity levels, which could relate to further changes in fat mass and the development of chronic diseases such as diabetes and cardiovascular disease. It has also been observed in the present study that the high fat diet has resulted in increased blood glucose level and incidence of glycosuria for male, which might be related to obesity. Development of obesity in animals eating high fat diet is the expected outcome, whether the animals are susceptible or resistant to obesity when eating high fat diets has a strong genetic component (West and York, 1998). Some strains of mice (A/J and C57/BL) and rats (Osborne-Mendel rat) are exquisitely susceptible to developing obesity when eating high fat or high carbohydrate diets, while other strains of mice (SWR) and rats (S5B/PI) are resistant to develop obesity when fed similar diets (George *et al.*, 2004). In addition, Rebuffe *et al.* (1993) and Surwit *et al.* (1995) reported that the same result in the B6 mouse is characterized by selective deposition of fat in the mesentery. It was observed that the male mice fed high fat diet developed diabetes and dysfunction of glucose metabolism. But the cause underlying this phenotype in Alloxan- induced diabetic-susceptible mice strain is not clear yet. Further studies are needed to elucidate the physiological functions of insulin and its role in metabolic disorders fatness.

It can be concluded that animals fed the high fat diet had significantly increased body weight and blood glucose levels compared with animals fed the control diet in both the sexes. The high fat diet male mice produced significantly higher perinephrium fat, mesenteric fat and weight of liver than those for control diet male mice. Results indicated that high fat diet might develop diabetes and dysfunction of glucose metabolism in male mice.

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