SMXA-5 Mouse as a Diabetic Model Susceptible to Feeding a High-fat Diet

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The SMXA-5 strain, a new mouse model for type 2 diabetes, is a recombinant inbred strain derived from non-diabetic SM/J and A/J strains. As dietary fat is a key component in the development of diabetes, we compared the glucose tolerance and diabetes-related traits among the SMXA-5, SM/J, and A/J strains while feeding a high-fat diet for 10 weeks. SMXA-5 fed on a high-fat diet showed an increased serum insulin concentration. Judging from the hyperinsulinemia in SMXA-5, this strain showed insulin resistance, an inability of peripheral tissues to respond to insulin, which was strengthened by feeding with a high-fat diet. When fed on a high-fat diet for 5 weeks, the SMXA-5 mice showed severely impaired glucose tolerance. On the other hand, SM/J showed mildly impaired glucose tolerance, even when fed on a high-fat diet for 10 weeks. These results indicate that SMXA-5 would be available for use as a diabetic model susceptible to a high-fat diet.

Key words: impaired glucose tolerance; high-fat diet; type 2 diabetes; mouse model

Type 2 diabetes is a multifactorial disease influenced by genetic and environmental factors. Environmental factors such as a typical western diet, in which more than 50% of the calories are derived from fat, increase the risk of developing type 2 diabetes. The heterogeneity of this disease, caused by the interaction of genetic and environmental factors, makes it difficult to understand the disease mechanism in human diabetes. Therefore, a diabetic animal model, especially one using inbred strains of mice, would be a valuable tool for exploring this heterogeneity, because genetic factors can be strictly controlled. As an environmental factor, dietary fat is a key component that influences the metabolic pathways involved in the development of diabetes. It has been reported that some inbred strains of mouse differentially develop diabetes and obesity in response to feeding with a high-fat diet. Increasing dietary fat causes insulin resistance and an impairment of insulin secretion. Recent progress in the genetic analysis of the mouse has enabled us to clarify the diabetogenic genes interacting with dietary factors. The development of diabetic strains susceptible to feeding with a high-fat diet is one approach to identifying the diabetogenic genes interacting with dietary fat.

The SMXA-5 mouse strain was recently introduced by Anunciado et al. as a new model for polygenic type 2 diabetes which moderately develops impaired glucose tolerance and hyperinsulinemia. SMXA-5 is one of the 26 SMXA recombinant inbred (RI) substrains that have been established from SM/J and A/J parental strains. Each of the RI strains has a different combination of parental genomes in a homozygous state. Although the SM/J and A/J strains are nondiabetic, SMXA-5 shows impaired glucose tolerance and hyperglycemia, suggesting that the interaction of latent diabetogenic genes derived from both non-diabetic parental strains leads to diabetes in SMXA-5. In the present study, we compare the glucose tolerance and diabetes-related traits among the SMXA-5, SM/J, and A/J strains fed on either a control or high-fat diet to evaluate the diabetes susceptibility of SMXA-5 under the influence of a high-fat diet.

Male mice of SM/J, A/J, and SMXA-5 strains (6-week-old) were provided by The Institute for Laboratory Animal Research (Nagoya University School of Medicine, Nagoya, Japan) and The Institute for Experimental Animals (Hamamatsu University School of Medicine, Hamamatsu, Japan), and were fed on a control or a high-fat diet ad libitum for 11 weeks. The composition (g/kg of diet) of the control diet was as follows: casein, 150; carbohydrate (starch/sucrose, 1:1), 728; AIN93MX mineral mixture, 35; AIN93VX vitamin mixture, 3); 10; choline chloride, 2; corn oil, 35; cellulose powder (AVICEL type FD-101, Asahi Chemical Industry, Osaka, Japan.), 40. The composition (g/kg of diet) of the high-fat diet was as follows: casein, 209; carbohydrate (starch/sucrose, 1:1), 728; AIN93MX mineral mixture, 35; AIN93VX vitamin mixture, 3); 10; choline chloride, 2; corn oil, 35; lard, 300; cellulose powder, 40. According to the report by Yoshida et al., the protein

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The non-fasting blood glucose concentration in the SMXA-5 mice was higher than that in the SM/J or A/J mice (Tables 1 and 2). In respect of the serum insulin concentration, a significant interactive effect between strain and diet was observed by two-way ANOVA (Table 2). In the mice fed on the high-fat diet, the serum insulin concentration was significantly higher than that in either SM/J or A/J. The final body mass index of SMXA-5 did not differ from that of A/J, but the final body weight of SMXA-5 mice was significantly lower than that of A/J, but higher than that of SM/J (Tables 1 and 2). The final body mass index of the three strains fed on the high-fat diet was higher than that of these strains fed on the control diet. Although data are not shown, the daily food intake relative to body weight (g/kg of body weight) of the SMXA-5 strain was no different from the value of the SM/J or A/J strain by both the 5th and 10th weeks. The liver weight (g/100 g of body weight) of the SMXA-5 strain was higher than that in either SM/J or A/J, irrespective of the diet. In the SMXA-5 mice, the weight of subcutaneous fat was higher than that in either SM/J or A/J. The subcutaneous fat and visceral fat weight in all strains fed on the high-fat diet were higher than those fed on the control diet (Tables 1 and 2). Adipose tissue produces and secretes a number of hormones (adipocytokines), including leptin, TNF-α, resistin, and adiponectin, which influence the insulin action in peripheral tissues. In particular, visceral fat accumulation is a risk factor for the impairment of glucose tolerance in cases of human type 2 diabetes. As the serum levels of adipocytokines might have been increased in the SMXA-5 animals, we are planning to measure these levels in future studies.

The non-fasting blood glucose concentration in the SMXA-5 mice was significantly lower than that of A/J, but higher than that of SM/J (Tables 1 and 2). When a significant effect of strain was observed without any interaction effect, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of the three strains (P < 0.05 being significant).

The initial body weight of the SMXA-5 mice was significantly lower than that of A/J, but higher than that of SM/J (Tables 1 and 2). In the case of IPGTT, the data were analyzed by three-way ANOVA, differences with P < 0.05 being regarded as significant. If the interaction effect of three components (strain × diet × feeding period) was significant by three-way ANOVA, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of all groups (P < 0.05, significant). When a significant effect of strain was observed without any interaction effect, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of the three strains (P < 0.05 being significant).

Data were analyzed by two-way ANOVA. When the interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of all groups (P < 0.05). When a significant effect of strain was observed without any interaction effect, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of the three strains (P < 0.05) shown in Table 2.

$^{abcd}$ Values in the same line not sharing a common superscript letter are significantly different at P < 0.05 by the Tukey-Kramer test.

| Table 1. Body Weight, Body Mass Index, Body Composition, and Serum and Liver Components of the SM/J, A/J, and SMXA-5 Strains Fed on the Control and High-fat Diets for 11 Weeks$^1$ |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Control diet    | High-fat diet   |
| Initial Body weight (g) | 13.5 ± 0.6      | 20.7 ± 0.6     | 17.7 ± 0.4      | 13.6 ± 0.4      | 21.6 ± 0.9     | 18.5 ± 0.5      |
| Final Body weight (g)    | 23.6 ± 1.0      | 32.7 ± 1.0     | 31.7 ± 1.1      | 24.8 ± 0.8      | 34.5 ± 1.2     | 35.6 ± 0.5      |
| Body mass index (g/cm$^2$) | 0.268 ± 0.007   | 0.297 ± 0.006  | 0.327 ± 0.009   | 0.280 ± 0.007   | 0.314 ± 0.010  | 0.364 ± 0.007   |
| Weights of tissue (g/100 g of body weight) | | | | | | |
| Liver             | 4.39 ± 0.11$^{ab}$ | 4.75 ± 0.07$^{bc}$ | 5.66 ± 0.29$^{ab}$ | 4.00 ± 0.13$^{bc}$ | 3.72 ± 0.09$^{bc}$ | 6.22 ± 0.35$^{ab}$ |
| Subcutaneous fat$^2$ | 1.87 ± 0.18      | 2.27 ± 0.12     | 3.72 ± 0.13      | 2.46 ± 0.34      | 3.08 ± 0.16     | 4.52 ± 0.18      |
| Visceral fat$^3$   | 1.49 ± 0.16$^{a}$ | 2.66 ± 0.15$^{bc}$ | 3.14 ± 0.13$^{ab}$ | 2.38 ± 0.22$^{a}$ | 3.43 ± 0.17$^{ab}$ | 3.29 ± 0.17$^{ab}$ |
| Serum glucose concentration (ng/ml) | 169 ± 8         | 173 ± 6         | 203 ± 11         | 167 ± 9         | 212 ± 12        | 212 ± 7         |
| Serum insulin concentration (ng/ml) | 1.12 ± 0.12$^a$ | 1.75 ± 0.19$^a$ | 5.00 ± 0.8$^a$   | 2.72 ± 0.5$^a$  | 2.45 ± 0.3$^a$  | 10.7 ± 2.2$^b$  |
| Liver triglycerides content (mg/100 g body weight) | 114 ± 19        | 179 ± 13        | 318 ± 7          | 166 ± 30        | 165 ± 22        | 482 ± 80        |

$^1$ Each value is expressed as the mean±SEM of 6 mice.

$^2$ Data were analyzed by two-way ANOVA. When the interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of all groups (P < 0.05). When a significant effect of strain was observed without any interaction effect, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of the three strains (P < 0.05) shown in Table 2.

$^{abcd}$ Values in the same line not sharing a common superscript letter are significantly different at P < 0.05 by the Tukey-Kramer test.

Per Calorie ratio of each diet was equalized (39.2 mg/Cal) to achieve optimum growth while feeding the high-fat diet. The body mass index was calculated as the body weight (g) divided by the square of the anal-nasal length (cm). Serum insulin concentration was measured by a radioimmunoassay (ShionoRIA, Shionogi, Osaka, Japan) with a rat insulin standard. An intraperitoneal glucose tolerance test (IPGTT) was performed in the 5th and 10th weeks of the experimental period. After a 14-hr fast, blood samples were collected from the tail vein (the fasting blood sample was the 0 min sample in IPGTT). A 20% glucose solution was then intraperitoneally injected (2 g of glucose/kg of body weight), and blood samples were collected 30, 60, and 120 min after the injection.\(^5\) The liver was stored at −20°C, and the triglyceride concentration in the liver was measured as described by Horio et al.\(^6\) The experimental data, except for the IPGTT data, were statistically analyzed by two-way ANOVA, a difference with P < 0.05 being regarded as significant. If the interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of all groups (P < 0.05 being significant). When a significant effect of strain was observed by two-way ANOVA without the interaction effect of two components, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of three strains (P < 0.05 being significant). In the case of IPGTT, the data were analyzed by three-way ANOVA, differences with P < 0.05 being regarded as significant. If the interaction effect of three components (strain × diet × feeding period) was significant by three-way ANOVA, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of all groups (P < 0.05, significant). When a significant effect of strain was observed without any interaction effect, one-way ANOVA and a subsequent Tukey-Kramer test were used to compare the means of the three strains (P < 0.05 being significant).
in SMXA-5 was higher than that in the SM/J or A/J strain. A significant increase in serum insulin concentration was observed only in the SMXA-5 mice fed on the high-fat diet, and was not seen in the SM/J and A/J strains fed on the high-fat diet (Tables 1 and 2). When fed on the control diet, the serum insulin concentration in SMXA-5 tended to be higher, but not significantly so, than that in the SM/J and A/J strains. Judging from the hyperinsulinemia in SMXA-5, this strain showed insulin resistance, an inability of peripheral tissues to respond to insulin, which was strengthened by feeding the high-fat diet. The hepatic triglyceride content was significantly higher in SMXA-5 than that in the SM/J and A/J mice (Tables 1 and 2). When fed on the control diet, the serum insulin concentration after 30 min during IPGTT (Fig. 1b). The blood glucose concentration after 120 min during IPGTT is recognized for the diagnosis of diabetes. This concentration in SMXA-5 fed on the high-fat diet was 489 mg/dl, 248 mg/dl in SM/J, and 239 mg/dl in A/J. When fed on the high-fat diet for 5 weeks, the SMXA-5 mice showed markedly impaired glucose tolerance and were diagnosed as diabetic. In the 10th week (Fig. 1d), the glucose tolerance curve for SMXA-5 or SM/J fed on the high-fat diet was similar to the respective one in the 5th week (Fig. 1b). In contrast, the blood glucose concentration during IPGTT of the A/J mice reached a peak value after 60 min, but not after 30 min, similar to the case of SMXA-5 (Fig. 1d). The glucose tolerance of A/J, but not SM/J, tended to deteriorate during the experimental period from the 5th to the 10th week. These results indicate that SMXA-5 developed severely impaired glucose tolerance in response to short-term feeding with the high-fat diet, and that SM/J was a diabetes-resistant strain that was not susceptible to being fed on the high-fat diet for only 5 weeks led to the development of severely impaired glucose tolerance in the three strains. In the 5th week, SMXA-5 fed on the high-fat diet showed a 200 mg/dl higher blood glucose concentration after 60 min than the value for SMXA-5 fed on the control diet, 130 mg/dl higher in SM/J, and 115 mg/dl higher in A/J (Figs. 1a and 1b). SMXA-5, but not SM/J or A/J, fed on the high-fat diet also showed the highest blood glucose concentration after 60 min during IPGTT (Fig. 1b). The blood glucose concentration after 120 min during IPGTT is recognized for the diagnosis of diabetes. This concentration in SMXA-5 fed on the high-fat diet was 489 mg/dl, 248 mg/dl in SM/J, and 239 mg/dl in A/J. When fed on the high-fat diet for 5 weeks, the SMXA-5 mice showed markedly impaired glucose tolerance and were diagnosed as diabetic. In the 10th week (Fig. 1d), the glucose tolerance curve for SMXA-5 or SM/J fed on the high-fat diet was similar to the respective one in the 5th week (Fig. 1b). In contrast, the blood glucose concentration during IPGTT of the A/J mice reached a peak value after 60 min, but not after 30 min, similar to the case of SMXA-5 (Fig. 1d). The glucose tolerance of A/J, but not SM/J, tended to deteriorate during the experimental period from the 5th to the 10th week. These results indicate that SMXA-5 developed severely impaired glucose tolerance in response to short-term feeding with the high-fat diet, and that SM/J was a diabetes-resistant strain that was not susceptible to being fed on the high-fat diet, in comparison with A/J and SMXA-5.

It has been reported that the C57BL/6J strain developed obesity and type 2 diabetes when weaned on to a high-fat diet. However, in this strain, the diabetic phenotype was observed after long-term (more than 16 weeks) feeding of the high-fat diet, and not with short-term feeding. In contrast, feeding the high-fat diet to the SMXA-5 mice for only 5 weeks led to the development of severely impaired glucose tolerance.

In conclusion, the SMXA-5 strain is a polygenic...
diabetic model with impaired glucose tolerance susceptible to feeding with a high-fat diet. In particular, short-term feeding with the high-fat diet to the SMXA-5 mice showed a marked difference in glucose tolerance from that of the parental SM/J and A/J strains.

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