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Characterization of diabetes-related traits in MSM and JF1 mice on high-fat diet

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Abstract

We examined the effect of a high-fat diet on the diabetes-related traits of the Japanese Fancy mouse 1 (JF1), MSM, and C57BL/6J (B6J) mice. MSM and JF1 mice were derived from *Mus musculus molossinus*. B6J is a commonly used laboratory strain, with the vast majority of genome segments derived from *Mus musculus domesticus* and *Mus musculus musculus*, and is susceptible to high-fat diet–induced type 2 diabetes. None of the strains showed symptoms of diabetes or obesity when fed a laboratory chow diet. Under a high-fat diet, JF1 mice developed impaired glucose tolerance, hyperglycemia, hyperinsulinemia, and obesity. B6J mice fed a high-fat diet mildly developed these diabetes-related traits compared to JF1 mice fed a high-fat diet. JF1 mice fed a high-fat diet were classified as having type 2 diabetes and were susceptible to high-fat diet–induced diabetes and obesity. On the other hand, MSM mice were resistant to high-fat diet–induced diabetes. These results indicate that the JF1 strain, with its unique genetic origin, is a useful new animal model of high-fat diet–induced diabetes and obesity. Further investigations using JF1 mice will help to clarify the role of the high-fat diet on human diabetes and obesity.

Keywords: High-fat diet; Type 2 diabetes; Obesity; JF1 mouse; MSM mouse; C57BL/6J mouse

1. Introduction

The worldwide incidence of diabetes has increased dramatically along with widespread lifestyle and dietary changes. Of particular importance may be proportion of fat in the diet. Diets high in fat are strongly associated with the development of obesity [1,2] and can induce insulin resistance in humans and animals [3–6]. It is clear that obesity constitutes a risk factor contributing to the development of type 2 diabetes. Type 2 diabetes, which accounts for more than 90–95% of all diabetes, is characterized by two metabolic defects: a deficiency of insulin secreted by the pancreatic β-cells and an inability of peripheral tissues to respond to insulin, a condition known as insulin resistance [7,8]. Both genetic and environmental factors play an important role in type 2 diabetes. Because this is a heterogeneous disease caused by a complex interaction of genetic and environmental factors, its mechanisms have proved to be elusive [9]. However, studies in appropriate animal models will provide additional insights into the physiological effects of specific susceptibility genes. Although a number of murine models for type 2 diabetes have been established [10–14], these models are insufficient for elucidating the complex and polygenic nature of this disease in humans. Most animal models for type 2 diabetes exhibit diabetic phenotype even when animals are given a stock chow diet [10–14], and their diabetic characteristics were largely examined while receiving a stock chow diet. In humans, genetic analyses and pathogenesis of obesity and diabetes caused by dietary factor such as fat are needed. At present, various animal models for diet-induced type 2 diabetes are required for developing this kind of study. The mouse model C57BL/6J (B6J) mouse is reported to be susceptible to high-fat diet–induced obesity and type 2 diabetes [15]. In addition, the susceptibilities to dietary obesity, but not diabetes, has been studied in nine inbred mouse strains (AKR/J, C57L/J, A/J, C3H/HeJ, DBA/2J, B6J, SJL/J, I/STN, and SWR/J) [16] fed a diet containing 36% of kilocalories as fat (condensed milk). Several quantitative

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trait loci (QTLs) were mapped by crosses between the high-fat diet–induced obesity susceptible strains and resistant strains [17]. However, more animal models for diet-induced diabetes and obesity are still needed for the reason that, in humans, these diseases are complex and polygenic in nature.

In addition, these laboratory mouse models have limited genetic heterogeneity, since the majority of laboratory strains originate from a small number of mice [18,19]. The genomes of laboratory strains such as B6J are mosaics, with the vast majority of segments derived from *Mus musculus domesticus* (*M. m. domesticus*) and *Mus musculus musculus*(*M. m. musculus*) [20]. The Japanese wild mouse, *Mus musculus molossinus*(*M. m. molossinus*),has several genetic characteristics clearly distinguishable from the European wild mouse, *M. m. domesticus*. Therefore, the strains MSM/Ms [21] and JF1/Ms [22], which were established from *M. m. molossinus*, are powerful genetic resources for linkage analysis and positional cloning [23]. Characterizations of both behavior and pain perception have been performed using MSM and JF1 strains, which may contribute to identifying genes underlying these traits [24,25]. However, little is known about the glucose metabolism, particularly with respect to the phenotypic characteristics of diabetes-related traits, in these strains. Thus, we thought it necessary to provide fundamental data on these strains fed a stock chow diet and high-fat diet.

In this study, we investigated the diabetes-related traits of MSM and JF1 mice fed a high-fat diet, and compared them with those of B6J mice, a commonly used laboratory strain known to be susceptible to high-fat diet–induced type 2 diabetes and obesity [15]. Moreover, the possibility that MSM or JF1 mouse is a new animal model for high-fat diet–induced diabetes was examined.

2. Methods and materials

2.1. Animals and diets

Male MSM/Ms (MSM) and JF1/Ms (JF1) mice were obtained from the National Institute of Genetics (NIG) in Mishima, Japan. C57BL/6J (B6J) mice were purchased from SLC (Hamamatsu, Japan). All mice were maintained in a room with a controlled temperature of 23° ± 3°C and humidity of 55% ± 5% under a 12-hour light/dark cycle and were fed a commercial stock chow (CE2; CLEA Japan, Tokyo, Japan) until 6 weeks of age. The fat content of this chow was 44 g/kg diet. Mice were then fed a commercial stock chow (Chow) or a high-fat diet (HF) ad libitum for 11 weeks. Six male mice were used in each group. All experiments were performed during the 11-week feeding with the Chow or HF diet. The composition (g/kg diet) of the HF diet was as follows: casein (Sigma Aldrich Japan, Tokyo, Japan), 209; carbohydrate (starch/sucrose, 1:1), 369; AIN93MX mineral mixture [26], 35; AIN93VX vitamin mixture [26], 10; choline chloride, 2; corn oil, 35; lard, 300; cellulose powder (AVICEL type FD-101; Asahi Chemical Industries, Osaka, Japan.), 40. Food intake was measured at weeks 5 and 10. All procedures were performed in accordance with the Animal Experimentation Guidelines of Nagoya University.

2.2. Body mass index and weight of tissues

Body weight and anal–nasal length were measured in anaesthetized mice at weeks 5 and 10 of the experimental period. Body mass index was calculated as the body weight (g) divided by the square of the anal–nasal length (cm). The subcutaneous fat, retroperitoneal fat, and mesenteric fat were dissected and weighed at the end of experiment. Subcutaneous fat was defined as fat pads below the root of the forefoot on one side of the body, and visceral fat was defined as the sum of retroperitoneal fat and mesenteric fat.

2.3. Measurement of serum triglyceride, insulin, and leptin concentrations

Blood samples were obtained in nonfasted mice at week 10 of the experimental period. Serum was obtained from tail-vein blood samples by centrifugation at 1600 × *g* and stored at −30°C until assay. The serum triglyceride was measured by the glycerol-3-phosphate oxidase method using a Triglyceride-E kit (WAKO, Osaka, Japan). Immunoreactive insulin concentration was measured by radioimmunoassay (ShionoRIA; Shionogi, Osaka, Japan) with rat insulin used as a standard. Serum leptin concentration was measured by enzyme-linked immunoassay (ELISA) using a mouse leptin ELISA kit (Morinaga, Yokohama, Japan).

2.4. Intraperitoneal glucose tolerance test and blood glucose concentration

The intraperitoneal glucose tolerance test (IPGTT) was performed at 5 and 10 weeks after the start of the Chow or HF diet. After 13–14 hours of fasting, blood samples were collected at 0 minute (fasting blood glucose) from the tail vein of each mouse. Each mouse was then intraperitoneally injected with 20% glucose solution (2 g/kg body weight), and 30 and 120 minutes after injection additional blood samples were collected. Blood glucose concentration was measured by the glucose oxidase method using a Glucose-B kit (WAKO, Osaka, Japan). Impaired glucose tolerance was defined as a blood glucose concentration >11.1 mmol/L at 120 minutes.

2.5. Insulin tolerance test

The insulin tolerance test (ITT) was performed at week 10 of the experimental period. This test was done only in mice fed a high-fat diet. After 13–14 hours of fasting, blood samples were collected at 0 minute (fasting blood glucose)

Table 1
Body weight, BMI, and food intake of MSM, B6J, and JF1 mice fed chow or high-fat (HF) diets for 5 or 10 weeks

	Chow			HF		
	MSM	B6J	JF1	MSM	B6J	JF1
5 Weeks						
Body weight (g)	11.5 ± 0.2^{a}	24.7 ± 0.7^{b}	16.4 ± 0.6^{c}	11.1 ± 0.2^{a}	26.4 ± 0.8^{b}	21.6 ± 0.6^{d}
BMI (g/cm^2)	0.176 ± 0.002^{a}	0.266 ± 0.003^{bc}	0.238 ± 0.005^{b}	0.180 ± 0.005^{a}	0.286 ± 0.004^{c}	0.318 ± 0.009^{d}
Food intake (g/g body weight/day)	0.335 ± 0.036^{a}	0.231 ± 0.015^{b}	0.212 ± 0.014^{bc}	0.145 ± 0.002^{c}	0.145 ± 0.006^{c}	0.149 ± 0.015^{c}
10 Weeks						
Body weight (g)	12.2 ± 0.4^{a}	26.4 ± 0.8^{bc}	17.3 ± 0.6^{a}	12.6 ± 0.4^{a}	31.1 ± 0.9^{b}	25.5 ± 1.0^{c}
BMI (g/cm^2)	0.181 ± 0.003^{a}	0.279 ± 0.005^{b}	0.227 ± 0.005^{c}	0.203 ± 0.003^{ac}	0.319 ± 0.008^{d}	0.338 ± 0.008^{d}
Food intake (g/g body weight/day)	0.288 ± 0.017^{a}	0.189 ± 0.009^{b}	0.175 ± 0.006^{bc}	0.133 ± 0.013^{cd}	0.124 ± 0.008^{d}	0.086 ± 0.005^{e}

Values are expressed as mean ± SEM of six mice. Data were analyzed by two-way ANOVA. When interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of all groups ($P < 0.05$) (shown in Tables 1 and 3). Values in the same line not sharing a common superscript letter are significantly different at $P < 0.05$ by Tukey-Kramer test.

from the tail vein of each mouse. Each mouse was then intraperitoneally injected with human insulin (Humulin R-40U; Eli Lilly Japan, Kobe, Japan) (0.25 U/kg body weight), and blood samples were collected at 30 and 60 minutes after the injection. Blood glucose concentrations were measured by the glucose oxidase method using a Glucose-B kit (Wako, Osaka, Japan).

2.6. Liver and skeletal muscle triglyceride concentrations

Liver and gastrocnemius muscle were removed at the end of the experiment and stored at −20°C until assay. The stored tissues were then homogenized with chloroform: methanol (2:1). A portion of this extract was dried, and the triglycerides contents of liver and skeletal muscle were measured by the glycerol-3-phosphate oxidase method using a Triglyceride-E kit (WAKO, Osaka, Japan).

2.7. Statistical analysis

All results are expressed as the mean ± SEM. The experimental data, except ITT data, were statistically analyzed by two-way analysis of variance (ANOVA). Differences with $P < 0.05$ were regarded as significant. If the interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and 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 by two-way ANOVA without interaction effect of two components, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of three strains ($P < 0.05$, significant). In the case of ITT, data were analyzed by one-way ANOVA. If effect of strain was significant by one-way ANOVA, the Tukey-Kramer test was subsequently used to compare the means of three groups ($P < 0.05$, significant). All statistical analyses were performed using StatView version 5.0 software (SAS Institute, Cary, NC).

3. Results

3.1. Body weight, body mass index, and food intake

Table 1 shows the body weight, body mass index (BMI), and food intake in MSM, B6J, and JF1 mice at weeks 5 and 10 (11 and 16 weeks of age) in the Chow and HF groups. Body weight in MSM or JF1 was significantly smaller than that in B6J at weeks 5 and 10, irrespective of diet (Tables 1 and 2). At week 5, only JF1 mice fed a high-fat diet, but not MSM mice and B6J fed a high-fat diet, showed a difference in body weight and BMI compared to their chow-fed counterparts. At week 10, BMI in JF1 and B6J mice, but not MSM mice, fed a high-fat diet were higher than their chow-fed counterparts. BMI of JF1-HF and B6J-HF (0.338 ± 0.008 g/cm^2 and 0.319 ± 0.008 g/cm^2, respectively) were significantly higher than that in MSM-HF (0.203 ± 0.003 g/cm^2). Although BMI in JF1-Chow was significantly lower than that in B6J-Chow, BMI in JF1-HF was comparable to that in B6J-HF.

Food intake (g/g body weight/day) in MSM-Chow was higher than that in B6J-Chow or JF1-Chow at weeks 5 and 10 (Table 1 and 2). Food intake did not differ significantly between B6J and JF1 mice fed a chow diet. Although, in mice fed a high-fat diet, there were no differences in food intake among the three strains at week 5, food intake in JF1 mice was significantly lower than that in MSM or B6J mice at week 10.

3.2. Serum insulin, leptin, lipids, and tissue weight

Table 3 shows the non-fasting serum insulin concentration, serum leptin concentration, serum triglycerides concentration, and weights (g/100 g body weight) of subcutaneous fat and visceral fat in MSM, B6J, and JF1 mice at week 11. In mice fed a chow diet, there were no significant differences in serum insulin concentrations among the three strains. B6J-HF and JF1-HF mice showed a significantly higher serum insulin concentration than did MSM-HF (Ta-

Table 2
Statistical analysis data on the effects of strain and diet on body weight, body mass index, food intake, serum components, and body composition

	P-value (two-way ANOVA)			Results of Tukey-Kramer test		
	Strain	Diet	Strain × Diet	MSM	B6J	JF1
5 Weeks						
Body weight	<0.001	<0.001	<0.001	–	–	–
BMI	<0.001	<0.001	<0.001	–	–	–
Food intake	0.002	<0.001	0.048	–	–	–
10 Weeks						
Body weight	<0.001	<0.001	0.002	–	–	–
BMI	<0.001	<0.001	<0.001	–	–	–
Food intake	<0.001	<0.001	<0.001	–	–	–
AUC: IPGTT	0.055	<0.001	0.028	–	–	–
Blood glucose concentration	0.012	<0.001	0.011	–	–	–
Serum insulin concentration	<0.001	<0.001	0.002	–	–	–
Serum TG concentration	0.032	NS	NS	A	B	AB
Serum leptin concentration	<0.001	<0.001	<0.001	–	–	–
Subcutaneous fat	<0.001	<0.001	0.010	–	–	–
Visceral fat	0.002	<0.001	NS	A	AB	B

Data were analyzed by two-way ANOVA. When interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of all groups ($P < 0.05$) (shown in Tables 1 and 3). When a significant effect of strain was observed without interaction effect, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of three strains ($P < 0.05$) (shown in Table 2). Values in the same line not sharing a common superscript letter are significantly different at $P < 0.05$ by Tukey-Kramer test.
NS = not significant ($P > 0.05$); TG = triglycerides.

bles 2 and 3). Serum triglyceride concentrations in three strains were not affected by a high-fat diet compared to a chow diet. Although serum leptin concentrations were significantly higher in high-fat diet–fed mice than in chow diet–fed mice of all three strains, the serum leptin concentration in JF1-HF mice was significantly higher than those in MSM-HF. Subcutaneous fat weight (g/100 g body weight) was significantly higher in high-fat diet–fed mice than in chow-fed mice of all three strains, subcutaneous fat weight in JF1 mice were significantly higher than those in MSM and B6J mice, irrespective of diet. Visceral fat weight in JF1 was higher than that in MSM and comparable to that in B6J (Tables 2 and 3).

3.3. Glucose tolerance

Figure 1A and 1B show the results of the glucose tolerance test at week 10 in MSM, B6J, and JF1 mice fed a chow diet and high-fat diet, respectively. As shown in Fig. 1A, there were no remarkable differences in the blood glucose concentrations during IPGTT among the three strains when fed a chow diet. None of the strains showed impaired glucose tolerance when fed a chow diet. In contrast, the blood glucose concentrations at 30 minutes of IPGTT in HF-fed mice of all three strains were significantly higher than those of their chow-fed counterparts (Fig. 1B). The concentration at 30 minutes in JF1-HF tended to be higher

Table 3
Serum components and body compositions of MSM, B6J, and JF1 mice fed chow or high-fat diets for 11 weeks

	Chow			HF		
	MSM	B6J	JF1	MSM	B6J	JF1
Areas under IPGTT curves (mmol · min/L)	1705 ± 114^{ab}	1540 ± 166^{a}	1758 ± 198^{ab}	2200 ± 134^{bc}	2466 ± 71^{c}	3101 ± 169^{d}
Blood glucose concentration (mmol/L)	9.45 ± 0.26^{a}	7.68 ± 0.24^{b}	9.00 ± 0.44^{a}	9.72 ± 0.35^{a}	11.00 ± 0.09^{c}	12.10 ± 0.63^{c}
Serum insulin concentration (pmol/L)	143 ± 31^{a}	152 ± 36^{a}	238 ± 33^{ab}	179 ± 38^{a}	491 ± 48^{b}	852 ± 140^{b}
Serum TG concentration (mmol/L)	1.91 ± 0.26	1.65 ± 0.15	1.60 ± 0.02	1.64 ± 0.08	1.11 ± 0.08	1.77 ± 0.14
Serum leptin concentration (mmol/L)	81 ± 16^{a}	266 ± 32^{a}	438 ± 58^{ab}	681 ± 113^{b}	1719 ± 174^{c}	2286 ± 238^{c}
Tissue weight (g/100 g body wt)						
Subcutaneous fat	1.12 ± 0.28^{ab}	1.09 ± 0.13^{a}	3.93 ± 0.42^{c}	2.54 ± 0.29^{d}	2.31 ± 0.20^{bd}	6.84 ± 0.27^{e}
Visceral fat	0.40 ± 0.11	0.96 ± 0.10	1.49 ± 0.23	1.49 ± 0.11	2.39 ± 0.18	3.01 ± 0.10

Values are expressed as mean ± SEM of six mice. Data were analyzed by two-way ANOVA. When interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of all groups ($P < 0.05$) (shown in Tables 2 and 3). When a significant effect of strain was observed without interaction effect, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of three strains ($P < 0.05$) (shown in Table 2). Values in the same line not sharing a common superscript letter are significantly different at $P < 0.05$ by Tukey-Kramer test.
TG = triglycerides.

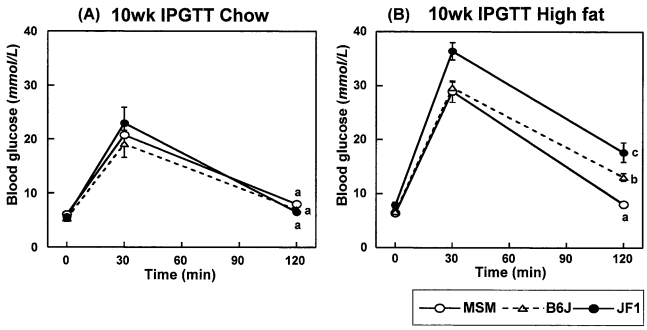

Fig. 1. Glucose tolerance test in MSM, B6J, and JF1 mice. Each mouse was fed (*A*) a chow diet or (*B*) high-fat diet for 10 weeks. Each value is expressed as the mean ± SEM of six mice. Data were analyzed by two-way analysis of variance (ANOVA) at each time point. When the interaction effect of two components (strain × diet) was significant by two-way ANOVA, one-way ANOVA and 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 an interaction effect, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of three strains ($P < 0.05$). At 0 minute in IPGTT the following values were observed: strain effect, NS; diet effect, $P = 0.019$; strain × diet interaction, NS. At 30 minutes in IPGTT: strain effect, NS; diet effect, $P < 0.001$; strain × diet interaction, NS. At 120 minutes in IPGTT: strain effect, $P = 0.004$; diet effect, $P < 0.001$; strain × diet interaction, $P < 0.001$. [abc]Means in the each time point not sharing a common superscript letter are significantly different by Tukey-Kramer test($P < 0.05$). NS = not significant ($P > 0.05$).

than those in MSM-HF and B6J-HF. At 120 minutes of IPGTT, the blood glucose concentrations in B6J and JF1 mice fed a high-fat diet were significantly higher than those in Chow-fed counterparts. The concentration of JF1-HF was significantly higher than that of B6J-HF. The blood glucose concentration at 120 minutes of IPGTT in MSM-HF was not different from the value in MSM-Chow (Fig. 1). The areas under the glucose tolerance curve (AUC:gtt) are also an appropriate value to assess the IPGTT data. By using the data of IPGTT curve, the areas under the glucose tolerance test curves (AUC:gtt) were calculated in each group (Table 3). On the chow diet, AUC:gtt was not different among the three strains (Table 3). AUC:gtt in B6J and JF1 mice fed a high-fat diet were significantly higher than those in chow-fed counterparts, but not in MSM mice. The value of JF1-HF was significantly higher than those of MSM-HF and B6J-HF.

3.4. Blood glucose concentrations

The nonfasting blood glucose concentrations at week 10 for chow-fed and HF-fed mice of all three strains were shown in Table 3. In B6J and JF1 mice, this concentration was significantly increased by feeding with a high-fat diet compared to feeding with a chow diet, but not in MSM mice. The blood glucose concentration of JF1-HF or B6J-HF was significantly higher than that in MSM-HF.

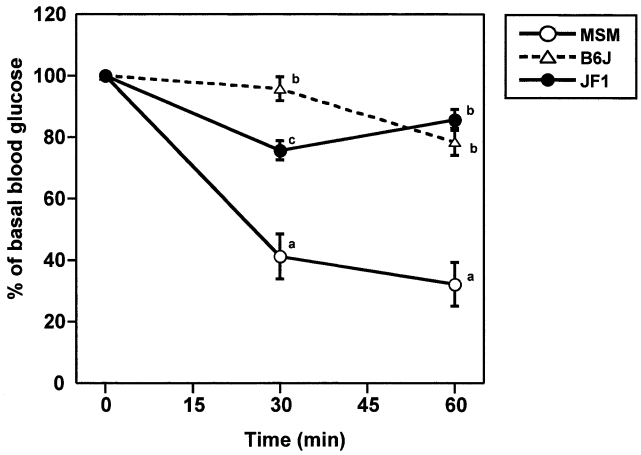

Fig. 2. Insulin tolerance test in MSM, B6J, and JF1 mice. Each mouse was fed high-fat diet for 10 weeks. Each value is expressed as the mean ± SEM of six mice. Data were analyzed by one-way analysis of variance (ANOVA) at each time point. One-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of all groups ($P < 0.05$). [abc]Means in the each time point not sharing a common superscript letter are significantly different ($P < 0.05$).

3.5. Insulin sensitivity

Figure 2 shows the results of the insulin tolerance test in mice fed a high-fat diet at week 10. The level of blood glucose at 30 and 60 minutes after the insulin injection in MSM, B6J, and JF1 mice is presented as a percentage of the respective fasting blood glucose concentration. The hypoglycemic response to exogenous insulin at both 30 and 60 minutes after the injection was significantly less in B6J and JF1 mice than in MSM mice. The hypoglycemic response at 30 minutes in JF1 mice was greater than that in B6J mice. However, the hypoglycemic response at 60 minutes in B6J mice was comparable to that in JF1 mice.

3.6. Triglyceride concentrations in liver and skeletal muscle

Figure 3 shows triglyceride concentrations in the liver and skeletal muscle. There were no significant differences among strains in the effect of diet on hepatic triglyceride concentrations. That is, feeding with a high-fat diet elevated hepatic triglycerides concentrations equally in all three strains compared to feeding with a chow diet. Skeletal muscle triglyceride concentrations in B6J were comparable to that in MSM. Skeletal muscle triglyceride concentrations in JF1 mice were markedly higher than that in MSM or B6J mice (Fig. 3B).

4. Discussion

The present study characterized diabetes-related traits in MSM and JF1 mice fed a high-fat or chow diet. MSM, JF1, and B6J mice did not show diabetic phenotypes and obesity

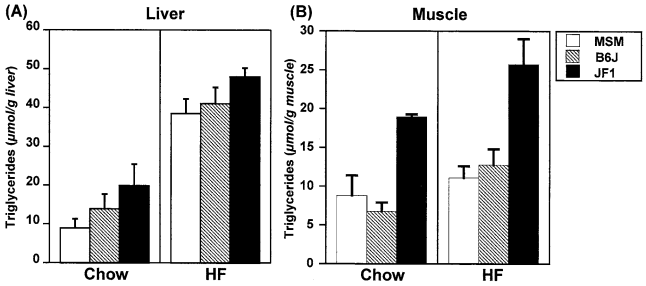

Fig. 3. Hepatic triglyceride concentrations (*A*) and muscle triglyceride concentrations (*B*) in MSM, B6J, and JF1 mice fed a chow diet (Chow) or high-fat diet (HF) for 10 weeks. Each value is expressed as the mean ± SEM of six mice. Data were analyzed by two-way analysis of variance (ANOVA). When a significant effect of strain was observed without interaction effect, one-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of three strains ($P < 0.05$). Hepatic triglyceride concentrations (*A*): strain effect, NS; diet effect, $P < 0.001$; strain × diet interaction, NS. Muscle triglyceride concentrations (*B*): strain effect, $P < 0.001$; diet effect, NS; strain × diet interaction, NS. Results of Tukey-Kramer test in strain effect: MSM, a; B6J, a; JF1, b.[ab]Means not sharing a common superscript letter are significantly different at by Tukey-Kramer test ($P < 0.05$). NS = not significant ($P > 0.05$).

when fed a chow diet. Although there are significant differences in BMI among three strains fed a chow diet, these levels of BMI are not recognized to be obese. MSM mice did not show impaired glucose tolerance, hyperglycemia, and insulin resistance even when fed a high-fat diet. On the other hand, JF1 and B6J mice exhibited impaired glucose tolerance, hyperglycemia, insulin resistance, and obesity when fed a high-fat diet for 10 weeks. JF1 mice fed a high-fat diet developed type 2 diabetes, judging from the extent of hyperglycemia and impaired glucose tolerance. When fed a high-fat diet, JF1 mice showed more severe impaired glucose tolerance than B6J mice fed a high-fat diet. At week 10, JF1 mice and B6J clearly developed high-fat diet–induced obesity. These results revealed that JF1 mice were susceptible to high-fat diet–induced diabetes, similar to B6J mice, which have been regarded as a model for diet-induced obesity and type 2 diabetes [15]. In contrast, MSM mice were demonstrated to be resistant to high-fat diet–induced diabetes. Visceral fat weight in JF1 mice was higher than that in MSM, and comparable to B6J. In particular, JF1 mice tended to store subcutaneous fat pads rather than visceral fat pads. Food intakes relative to body weight in JF1 mice or B6J were not higher than that in MSM mice. From these results, it is supposed that energy expenditure is decreased in JF1 or B6J mice, leading to the greater BMI and fat accumulation. We also measured serum leptin concentration (Table 3), which suppresses food consumption and stimulates energy expenditure in peripheral tissues [27]. It has been reported that serum leptin concentrations are positively correlated with body weight or body mass index, and obese mice have been shown to have greater serum leptin levels than nonobese mice [28,29]. Therefore, it has been hypothesized that obese mice may be resistant to the actions of leptin. In this study, serum leptin concentrations were elevated by a high-fat diet in all three strains, and were positively correlated with the increase of visceral fat or subcutaneous fat weight rather than body weight or body mass index in the comparison among the three strains. Although serum leptin concentrations in all mice fed a high-fat diet differed from those fed with a chow diet, it remains uncertain whether an impairment of the actions of leptin was provoked in three strains by a high-fat diet.

The finding of hyperinsulinemia and decreased insulin sensitivity in JF1 mice fed a high-fat diet indicated the existence of insulin resistance. It was suggested that the impaired glucose tolerance in JF1 mice fed a high-fat diet (Fig. 1B) was due to insulin resistance and possibly to insulin secretion as well. The liver and skeletal muscle are the most important insulin-responsive organs in the body [30], and the relationship between the intracellular accumulation of fatty acid–derived metabolites and insulin resistance has been demonstrated using experimental models [31]. A strong correlation between accumulation of triglycerides in myocytes and insulin resistance has been reported [32,33]. Another study provided the noteworthy result that transgenic mice with skeletal muscle- or liver-specific overexpression of lipoprotein lipase had a 3- or 2-fold increase in triglycerides concentration in each tissue, respectively, and simultaneously showed remarkable insulin resistance [34]. As shown Fig. 3, the triglycerides concentration in the skeletal muscle in JF1 mice was about 2-fold higher than that in B6J or MSM mice, irrespective of diet. Nonfasting serum insulin concentration in JF1-Chow tended to be higher than those in MSM-Chow and B6J-Chow (Table 3). This result might imply slight insulin resistance not exhibiting impaired glucose tolerance in JF1-Chow. We suggested that the triglycerides accumulation in the skeletal muscle in JF1 mice was one of many causes in insulin resistance.

On the other hand, there were no significant differences in the hepatic triglycerides level between JF1 mice and either MSM or B6J mice. Thus, we speculate that the accumulation of triglycerides in the skeletal muscle was one of the causes of the insulin resistance observed in JF1 mice. Fat-derived circulating hormones such as tumor necrosis factor–α, resistin, and adiponectin are known to play an important role in insulin resistance [35]. Serum levels of these adipocytokines should be measured in JF1, B6J, and MSM mice in a future study.

As mentioned previously, the JF1 strain was established from *M. m. molossinus*, and the genomes of laboratory strains are mosaics, with vast majority of segments derived from *M. m. domesticus* and *M. m. musculus*. Therefore, JF1 mouse is highly useful for genetic mapping because of the high level of polymorphisms between *M. m. molossinus* and laboratory strains. JF1 strain developed high-fat diet–induced type 2 diabetes at early stage of age compared to B6J strain. For these reasons, by crossing the JF1 strain and

another resistant laboratory strain, we can efficiently dissect the genetic factors causing type 2 diabetes and obesity. Moreover, JF1 strain might have unique genetic factors causing high-fat diet–induced type 2 diabetes. This study revealed that JF1 mouse, with their unique genetic origin, is a useful new animal model for high-fat diet–induced diabetes and obesity. Further investigations on JF1 mice will help to elucidate the relationship between the high-fat diet and human diabetes and obesity. On the other hand, MSM mice, which are resistant to a high-fat diet–induced diabetes, are readily available as a counterpart for genetic analysis, using crosses with a susceptible laboratory strain.

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