Selective breeding of mice for different susceptibilities to high fat diet-induced glucose intolerance: Development of two novel mouse lines, Selectively bred Diet-induced Glucose intolerance-Prone and -Resistant

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ABSTRACT

Aims/Introduction: The development of type 2 diabetes is primarily due to lifestyle and environmental factors, as well as genetics, as shown by familial clustering. To establish mouse lines for evaluating heritable factors determining susceptibility to diet-induced diabetes, we performed selective breeding for differences in high fat diet (HFD)-induced glucose intolerance.

Materials and Methods: Selective breeding was performed using hybrid mice of C57BL/6J, C3H/HeJ and AKR/N backgrounds. After 5-week HFD feeding, mice showing high and low 2-h blood glucose levels in an oral glucose tolerance test (OGTT) were selected and bred over 14 generations to produce lines prone and resistant to diet-induced glucose intolerance (designated Selectively bred Diet-induced Glucose intolerance-Prone [SDG-P] and -Resistant [SDG-R]).

Results: At 5 weeks of age (pre HFD feeding), SDG-P mice showed higher blood glucose levels both in the OGTT and insulin tolerance test as compared to SDG-R mice. After receiving HFD, the glucose intolerance of SDG-P mice became more evident without hyper insulin secretion. In addition, SDG-P mice had greater body weight gain and lower HDL-cholesterol levels as compared to SDG-R mice. In comparison with C57BL/6J, a well-known strain prone to HFD-induced glucose intolerance, SDG-P mice showed significantly higher glucose levels in OGTT after the 5-week HFD feeding.

Conclusions: Susceptibility to HFD-induced glucose intolerance was transmitted over generations and was intensified by selective breeding. The newly established mouse lines, SDG-P and SDG-R, may be useful in investigating the pathophysiology of type 2 diabetes through elucidating the crucial factors for determining the susceptibility to HFD-induced glucose intolerance.

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KEY WORDS: Dietary fats, Glucose intolerance, Mice

INTRODUCTION

The incidence of lifestyle-dependent diseases such as type 2 diabetes is increasing explosively around the world, as a result of modern environmental factors such as a Western diet (high-calorie foods with abundant fat) and sedentary lifestyles. Excess dietary fat consumption leads to insulin resistance, which in turn causes various metabolic disorders including type 2 diabetes, hypertension and dyslipidemia.

Individual differences in susceptibility to environmental influences such as a Western diet are postulated to be determined by hereditary factors. In humans, a short-term high fat diet (HFD) leads to more severe insulin resistance in individuals with a family history of type 2 diabetes. In rodents, the propensity for developing HFD-induced diabetes and obesity varies widely among strains. The C57BL/6 mouse strain is highly susceptible to HFD-induced diabetes and obesity, which has been determined to be due to an impaired insulin secretion pattern as compared with diabetes-resistant strains. Levin et al. reported that HFD-induced obesity-prone and -resistant traits in Sprague-Dawley rats could be developed in two distinct inbred lines by selective breeding.

Selective breeding has been used to develop several animal models for diabetes research. The polygenic background of selectively bred animal models mimics the human pathophysiology.
of type 2 diabetes. For example, the Goto-Kakizaki (GK) rat is a non-obese diabetic model derived from Wistar rats by repetitive selective breeding for impaired glucose metabolism. Diabetes in GK rats is a result of insulin secretion deficiency due to rapid β-cell loss with inflammatory changes in the islets, resembling the pathogenesis of human diabetes. Recently, Cummings et al. established an obese type 2 diabetes rat model (UCD-T2DM) by crossbreeding insulin-resistant obese Sprague-Dawley rats with insulin production-impaired ZDF-lean rats and subsequent selective breeding of the offspring for the obese phenotype.

To establish mouse lines for investigating heritable factors that determine susceptibility to diet-induced glucose metabolism impairment, we performed selective breeding of mice that displayed high and low blood glucose levels in an oral glucose tolerance test (OGTT) after HFD feeding. As a result, we established two distinct lines of mice with HFD-induced glucose intolerance-prone and glucose intolerance-resistant characteristics; designated Selectively bred Diet-induced Glucose intolerance-Prone (SDG-P) and -Resistant (SDG-R). These two lines of mice are expected to be useful models for investigating the pathophysiology of type 2 diabetes through elucidating the crucial factors that determine susceptibility to HFD-induced glucose intolerance.

**MATERIALS AND METHODS**

**Animals and Diets**
C57BL/6J (B6) and C3H/HeJ (C3H) mice were purchased from CLEA-Japan (Tokyo, Japan) and AKR/N (AKR) mice were obtained from Japan SLC (Hamamatsu, Japan). Mice were housed 3–5 animals per cage and maintained in a temperature-controlled room on a 12 h light/dark cycle with ad libitum access to water and standard rodent chow (MF; Oriental Yeast, Tokyo, Japan) unless otherwise specified. This study protocol was approved by the Nippon Medical School Animal Policy and Welfare Committee.

**Selective Breeding**

Figure 1 shows the genealogy chart of the SDG-P and SDG-R mice strains. To establish a novel mouse model for HFD-induced glucose metabolism impairment, we performed selective breeding with B6, C3H and AKR mice as background strains. Since some mice showed resistance to developing HFD-induced glucose intolerance during the selective breeding trial, we divided the strain into two groups as shown in Figure 1, i.e., the mice that showed high and low blood glucose levels at 120 min (BG120min) in the OGTT after 10 weeks of HFD feeding (Quick Fat; CLEA-Japan; providing 32% energy as fat) were selected as G1 of...
SDG-P and SDG-R, respectively. The selective breeding for high BG120min in the SDG-P and low BG120min in the SDG-R groups was repeated for subsequent generations. Due to a low birth rate, male G4 mice of both lines were crossed with B6 females and the HFD feeding period was shortened to 5 weeks (5–10 weeks of age) at G5.

**Oral Glucose Tolerance Test (OGTT)**

The OGTT was performed within 7 days after the end of the HFD feeding period. After overnight-fasted blood glucose levels were measured, glucose (2 g/kg body weight) was orally administered and blood glucose levels were measured at 30, 60 and 120 min after the administration with a glucose sensor using tail vein blood (2 μL). To evaluate the insulin response in OGTT, blood plasma was prepared by centrifugation of tail vein blood (20 μL) with EDTA as an anticoagulant. Plasma insulin levels were measured using an ultra-sensitive mouse insulin ELISA kit (Morinaga, Yokohama, Japan).

**Insulin Tolerance Test (ITT)**

After overnight-fasted blood glucose levels were measured, 1 U/kg body weight of insulin (Humalin R; Eli Lilly Japan, Tokyo, Japan) was injected intraperitoneally. Blood glucose levels were measured at 15, 30, 60 and 120 min after the injection, as described above.

**Lipid Analysis**

Plasma samples were prepared from tail vein blood (40 μL) as described above. Total cholesterol (TC), HDL cholesterol (HDL-C), triglycerides (TG) and nonesterified fatty acids (NEFA) were determined using colorimetric kits (Wako Pure Chemical, Osaka, Japan).

**Statistical Analysis**

Data are expressed as mean ± SEM. Mean values were compared using the Student’s t test and a P value of <0.05 was considered statistically significant. Statistical analyses were performed using JMP 9 (SAS Institute, Cary, NC, USA).

**RESULTS**

**Establishment of SDG-P and SDG-R Strains**

As a result of the selective breeding over 10 generations, the two lines of mice showed significantly different blood glucose responses in the OGTT after the 5-week HFD feeding. SDG-P mice showed marked glucose intolerance in the OGTT, whereas SDG-R mice maintained lower glucose levels (Figure 2a,b). The glucose intolerance of SDG-P mice was present in both males and females, with slightly higher glucose levels seen in male mice. During the HFD feeding period, SDG-P mice had a significantly higher body weight and a higher food intake as compared to SDG-R mice (Figure 2c–f). These characteristics of the mice were inherited over generations.

**Long-Term Characteristics of SDG-P and SDG-R Mice**

To examine the long-term profiles of SDG-P and SDG-R mice, males of each line (n = 10) were individually housed and followed up until 50 weeks of age. At 5 weeks of age (pre HFD feeding), SDG-P mice demonstrated modest but significantly higher blood glucose levels in an OGTT as compared to SDG-R mice (Figure 3a). This response became more evident at 10 weeks of age (post HFD feeding) and the marked glucose intolerance remained at 25 weeks of age, even after the diet was replaced with normal chow (Figure 3b,c). However, the impaired glucose tolerance in SDG-P mice was ameliorated at 50 weeks of age (Figure 3d).

Plasma insulin levels in both lines of mice gradually increased with age. Despite the different blood glucose responses in the OGTT, the glucose-stimulated insulin response was not significantly different between SDG-P and SDG-R mice at 5, 10 and 25 weeks of age, whereas the insulin response of 50-week-old SDG-P mice was markedly greater than that of age-matched SDG-R mice (Figure 3e–h).
In the ITT performed at 5 weeks of age (pre HFD feeding), SDG-P mice showed significantly higher blood glucose levels as compared to SDG-R mice (Figure 3i). The lower insulin sensitivity in SDG-P mice was also observed at all other time points of follow-up (Figure 3j–l).

Plasma lipid profiles are shown in Table 1. No significant differences in TC, HDL-C, TG and NEFA were observed between SDG-P and SDG-R mice at 5 weeks of age. After the HFD feeding, TC in 10-week-old SDG-P was lower than that in age-matched SDG-R mice, mainly due to their lower HDL-C levels.
No significant differences in lipid profiles were observed between both lines at 50 weeks of age.

Even after the diet was replaced with normal chow at 10 weeks of age, the body weight of SDG-P mice remained higher than that of SDG-R mice until 50 weeks of age (Figure 4a). Correspondingly, a trend toward hyperphagia in SDG-P mice was also observed throughout the follow-up period, as compared to age-matched SDG-R mice (Figure 4b).

Comparison of SDG-P and SDG-R With B6

Even when compared to the glucose intolerance-prone B6 mice, SDG-P mice showed significantly higher glucose levels in an OGTT after 5-week HFD feeding (Figure 5a). In contrast, the SDG-R mice showed lower glucose levels than B6 mice. During the HFD feeding period, SDG-P mice had a higher body weight than B6 mice, whereas no significant body weight differences were observed between SDG-R and B6 mice (Figure 5b).

DISCUSSION

Several animal models are currently used in the field of type 2 diabetes research. For instance, GK rats\textsuperscript{10} and NSY\textsuperscript{14} mice, both of which were produced by selective breeding with glucose tolerance as a selection index, are useful models to analyze the pathogenesis of type 2 diabetes, mainly due to $\beta$ cell failure\textsuperscript{11,14,15}. Additionally, $db/db$ mice\textsuperscript{16} and Zucker diabetic fatty rats\textsuperscript{17}, both of which are defective in leptin signaling, are also used for the study of the obesity-related type 2 diabetes with insulin...
resistance. However, these type 2 diabetes models could not explain the genetic factors for determining the susceptibility to diet-induced diabetes. In this study, we thus aimed to produce new animal models to explain the pathogenesis of diet-induced diabetes and established two distinct mice lines that have prone and resistant phenotypes to HFD-induced glucose intolerance by selective breeding. To our knowledge, this is the first report of the selective breeding study to enhance the susceptibility to HFD-induced glucose intolerance as hereditary phenotypes.

To establish our mice lines, we first produced F1 hybrid mice derived from B6 and C3H to diminish inbreeding depression in fertility. Nevertheless, it was necessary to outcross several times to maintain the fertility. We considered that the reduced litter size should be mainly due to maternal obesity because the 10-week HFD feeding induced severe obesity (data not shown). HFD-induced obesity has been reported to cause infertility in several rodent models. Therefore, we shortened the HFD feeding period to 5 weeks at G5 in order to maintain SDG-P and SDG-R mice with adequate fertility (Figure 1). Under the ongoing breeding protocol, adequate litter size has been observed stably in both lines to maintain the strains without further outcrosses (6–7 pups per litter on average, data not shown).

Among the background strains we used, B6 is the most prone to HFD-induced glucose intolerance, whereas AKR is prone to HFD-induced obesity with insulin resistance. Thus, their hybrids would vary more widely in their susceptibility to HFD-induced glucose intolerance. The heterogenic background enabled us to develop two distinct lines. As a result, even in comparison with B6, SDG-P mice showed more severe glucose intolerance after the HFD feeding (Figure 5). On the other hand, despite selecting mice with higher blood glucose levels until the F1 generation, glucose intolerance-resistant SDG-R mice were isolated from F2 (Figure 1). These results of selective breeding strongly suggest that the susceptibility to HFD-induced glucose intolerance is regulated by polygenic factors in SDG-P and SDG-R mice.

Similar to clinical pre-diabetes in humans, SDG-P mice showed modestly higher blood glucose levels in OGTT and reduced insulin sensitivity in ITT even prior to the HFD feeding period, suggesting that SDG-P mice mimic the pathogenesis of glucose metabolism impairment in humans. After receiving HFD feed, SDG-P mice showed higher blood glucose levels in OGTT. However, there were no differences in the insulin response between the two strains. After resuming the normal chow feed, a higher insulin response was observed and the glucose intolerance was ameliorated in SDG-P mice at 50 weeks of age. These results indicate that insulin secretory response to increased blood glucose was impaired in the islets of SDG-P mice during the HFD feeding, but slowly recovered when fed normal chow. In this study, HFD was fed only for 5 weeks to maintain fertility as aforementioned. However, further research is needed to evaluate whether long-term HFD feeding in SDG-P mice causes an irreversible impairment in insulin secretion and consequently overt hyperglycemia.

Although BG120min in OGTT was the sole criterion for selection, SDG-P mice showed not only glucose intolerance, but also a greater body weight gain and lower HDL-C levels after the 5-week HFD feeding period. The clustering of metabolic impairments suggests that SDG-P is a promising model for studying HFD-related metabolic abnormalities, because the co-occurrence of hyperglycemia, overweight, dyslipidemia and hypertension is often observed clinically. Further investigation into these metabolic abnormalities including blood pressure measurement in SDG-P mice may be helpful for understanding the underlying mechanism for the clustering of lifestyle-related metabolic abnormalities.

In summary, we established two distinct mice lines, SDG-P and SDG-R, which are prone and resistant, respectively, to HFD-induced glucose intolerance. The results of selective breeding indicate that the susceptibility to HFD-induced glucose intolerance is regulated by polygenic factors and transmitted over generations. The HFD-induced glucose intolerance prone SDG-P mice showed spontaneous whole body insulin resistance and inadequate insulin secretory response to HFD-induced blood glucose elevation. Thus, these factors may be critical for determining the susceptibility to HFD-induced glucose intolerance. Further studies on these factors, including the genetic or epigenetic mechanisms, may be meaningful for elucidating the pathogenesis of glucose intolerance and other lifestyle-related metabolic abnormalities.

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