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NOTE

Transgenerational changes of metabolic phenotypes in two selectively bred mouse colonies for different susceptibilities to diet-induced glucose intolerance

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Abstract. We recently established 2 mouse lines with different susceptibilities (prone and resistant) to high-fat diet (HFD)-induced glucose intolerance by selective breeding (designated selectively bred diet-induced glucose intolerance-prone [SDG-P] and -resistant [SDG-R], respectively). In the present study, we analyzed transgenerational changes in metabolic phenotypes in these 2 mouse colonies to explore how the distinct phenotypes have emerged through the repetitive selection. Using C57BL/6, C3H, and AKR as background strains, mice showing inferior and superior glucose tolerance after HFD feeding were selected and bred repetitively over 20 generations to produce SDG-P and SDG-R, respectively. In addition to the blood glucose levels, HFD intake and body weight were also measured over the selective breeding period. As the generations proceeded, SDG-P mice became more susceptible to HFD-induced glucose intolerance and body weight gain, whereas SDG-R mice had gradually reduced HFD intake. The differences in fasting and post-glucose challenge blood glucose levels, body weight, and HFD intake became more evident between the 2 colonies through the selective breeding, mainly due to the HFD-induced glucose metabolism impairment and body weight gain in SDG-P mice and the reduction of HFD intake in SDG-R mice. These transgenerational changes in the metabolic phenotypes suggest that the genetic loci associated with the quantitative traits have been selectively enriched in SDG-P and SDG-R.

Key words: High-fat diet, Type 2 diabetes, Selective breeding, Feeding behavior

SELECTIVE breeding is a powerful approach to investigate genotype-phenotype interactions in complex traits involving multiple genes. The polygenic background of selectively bred animal models can mimic the polygenic etiology of lifestyle-related diseases, such as type 2 diabetes and obesity. For instance, Goto-Kakizaki (GK) rat [1] and Nagoya-Shibata-Yasuda (NSY) mouse [2], both of which were selectively bred for hyperglycemia, are useful models to investigate the pathogenesis of type 2 diabetes. Levin *et al*. [3] performed repetitive selective breeding for diet-induced obesity-prone (DIO) and -resistant (DR) traits in Sprague-Dawley rats. Although body weight was the sole criterion for the selection, DIO rats also exhibited diet-induced hyperglycemia compared with DR rats [3, 4]. Similarly, other metabolic traits often accompany the selection criterion in selectively bred rodent models (*e.g.*, hyperphagia in young-adult GK rats [5], cardiovascular diseases in spontaneously hypertensive (SHR) rats [6]). Such a cluster of metabolic impairments in selective breeding models resembles the pathophysiology of type 2 diabetes and obesity in humans.

Recently, we established novel mouse models that mimic the gene-environment interactions in the development of type 2 diabetes by selective breeding [7]. In brief, using C57BL/6, C3H, and AKR as background strains, mice exhibiting both inferior and superior glucose tolerance after high-fat diet (HFD) feeding were selected and bred repetitively. Consequently, 2 mouse lines with distinctively different susceptibilities (prone and resistant) to HFD-induced glucose intolerance were established and designated *s*electively bred *d*iet-induced *g*lucose intolerance-*p*rone (SDG-P)

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and -*r*esistant (SDG-R), respectively. We previously demonstrated that SDG-P mice developed overt diabetes with mild obesity after 5 weeks of HFD feeding at the 10th–12th generations [7] and had hereditary defects in insulin secretion at the 15th–17th generations [8] as compared with SDG-R mice. Although these previous studies within the close generations would be useful for assessing phenotypic differences and the pathophysiological consequences between the 2 colonies of mice, we have to know the transgenerational changes to understand how the differences have emerged through the repetitive selection. During the selective breeding, we have serially measured HFD intake and body weight of all mice in addition to the blood glucose levels. Thus, in the present study, we analyzed the transgenerational changes in these metabolic phenotypes to clarify how the differences have emerged between the 2 colonies.

Materials and Methods

Selective breeding of SDG-P and SDG-R mice

The selective breeding of SDG-P and SDG-R mice was performed at Sunplanet Co. (Tokyo, Japan) until the 11th–12th generations and then at the Institute for Animal Reproduction (Kasumigaura, Japan) after the 12th–13th generations. During the 5th–20th generations, mice were fed an HFD (Quick Fat [32% energy from fat]; CLEA Japan, Tokyo, Japan) for 5 weeks (5–10 weeks of age) [7]. HFD intake was monitored every week, and daily food intake per mouse was calculated from the 5-week HFD consumption per cage (3–5 mice). Body weight was measured at the end of HFD feeding. Glucose tolerance was assessed by oral glucose tolerance test (OGTT) within 1 week after the end of the HFD feeding period as follows: after overnight fasting blood glucose (FBG) levels were measured, glucose (2 g/kg body weight) was administered orally and blood glucose levels were measured at 30, 60, and 120 min after the administration with a glucose sensor (Glutest Neo Super; Sanwa Kagaku Kenkyusho, Nagoya, Japan) by tail bleeding. These measurements (food intake, body weight, and blood glucose profiles in OGTT) were performed in all mice of each generation. Mice of both sexes showing higher and lower blood glucose levels at 120 min in OGTT ($BG_{120\ min}$) were selected to breed the next generations of SDG-P and SDG-R, respectively. The total number of mice in each generation and the number of pairs selected for breeding were shown in Supplementary Table S1 (the average number ± standard error of mean (SEM) of mating pairs from the 5th to 20th generation was 11.3 ± 0.8 and 8.1 ± 0.8 for SDG-P and SDG-R, respectively). The study was conducted with approval from the institutional animal care and use committees of Sunplanet Co, the Institute for Animal Reproduction, and Nippon Medical School.

Statistical analysis

Data are expressed as mean ± SEM. The parameter distribution in each generation is summarized as a box-and-whisker plot. Mean values were compared using the Student t test. A trend analysis was performed with Jonckheere–Terpstra trend test. Correlations of average differences between SDG-P and SDG-R in each generation with generation progress were evaluated by Pearson correlation analysis. $P < 0.05$ was considered as significant.

Results

Intergenerational transitions of FBG, $BG_{120\ min}$, post-HFD body weight, and HFD intake of all mice in each generation are shown in Fig. 1. As the generations proceeded, the selection index $BG_{120\ min}$ showed a marked increase in SDG-P mice, whereas it elevated slightly in SDG-R mice (Fig. 1c, d). In addition to $BG_{120\ min}$, FBG increased gradually in SDG-P mice of both sexes, whereas it decreased in female SDG-R mice (Fig. 1a, b). The area under curve of blood glucose levels in OGTT (BG_{AUC}) also increased in SDG-P mice (Supplementary Fig. S1).

Post-HFD body weight was increased in SDG-P mice of both sexes as the generations proceeded, whereas it decreased slightly in female SDG-R mice (Fig. 1e, f). Despite the increasing trend of body weight in SDG-P mice, the HFD intake was not altered in the male mice and even decreased in the female (Fig. 1g, h). In SDG-R mice, HFD intake was significantly decreased in both sexes (Fig. 1g, h).

Linear regression analysis revealed that differences in the average values of FBG, $BG_{120\ min}$, BG_{AUC}, post-HFD body weight, and HFD intake became more evident between SDG-P and SDG-R as the generations proceeded (Fig. 2 and Supplementary Fig. S2).

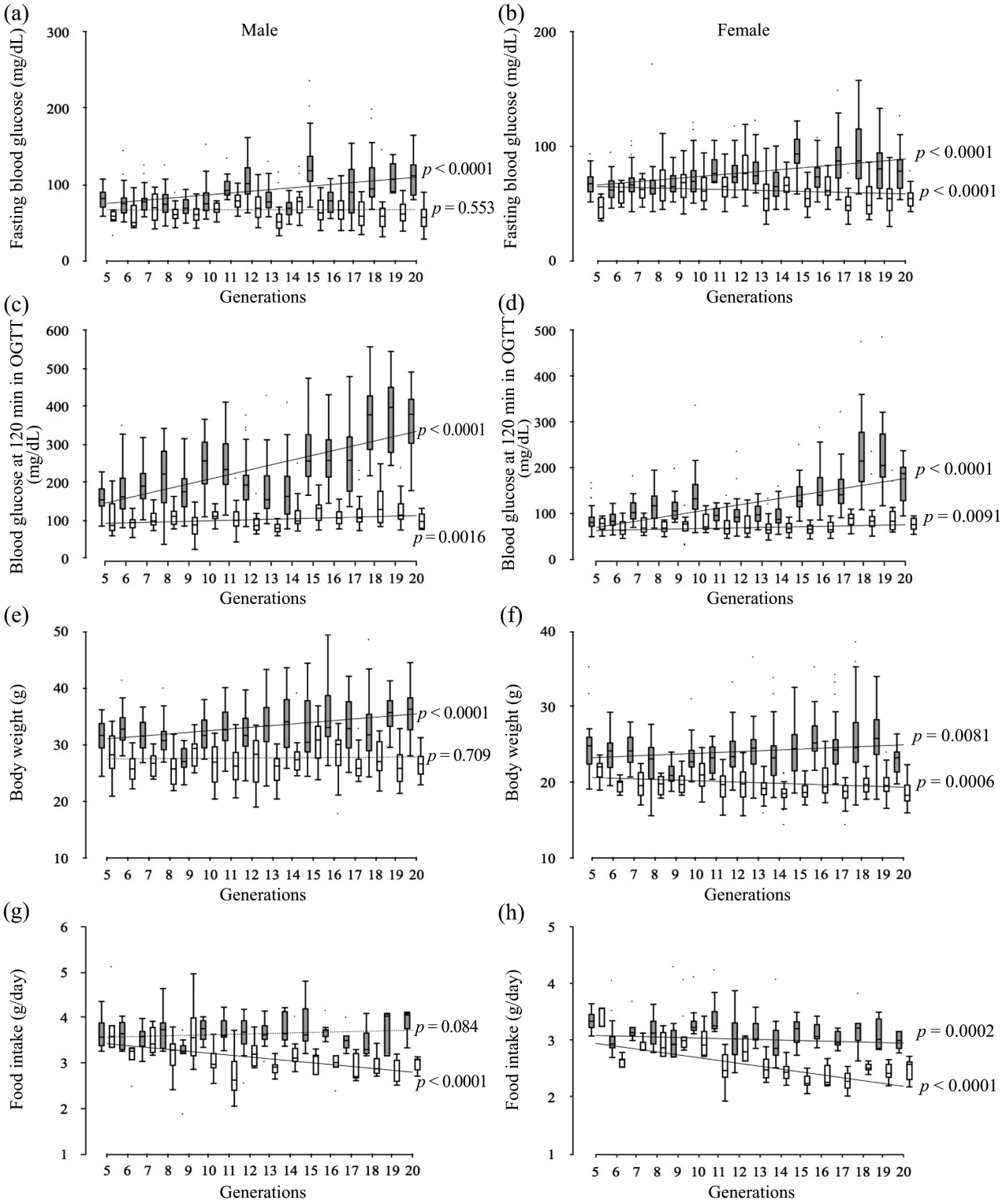

Fig. 1 Intergenerational distributions of metabolic phenotypes.
Fasting blood glucose (a, b), blood glucose at 120 min in OGTT (c, d), body weight after 5-week HFD feeding (e, f), and HFD intake (g, h) in SDG-P (gray boxes) and SDG-R (white boxes). The boxes plot the central rectangular spans from the first quartile to the third quartile (interquartile range; IQR) of each breeding generation. A segment inside the rectangle shows the median. The whiskers display the lowest datum within 1.5 IQR of the lower quartile and the highest datum within 1.5 IQR of the upper quartile. Data not included between the whiskers are plotted as outliers (dots). The *p* values by Jonckheere-Terpstra trend test are shown in each panel.

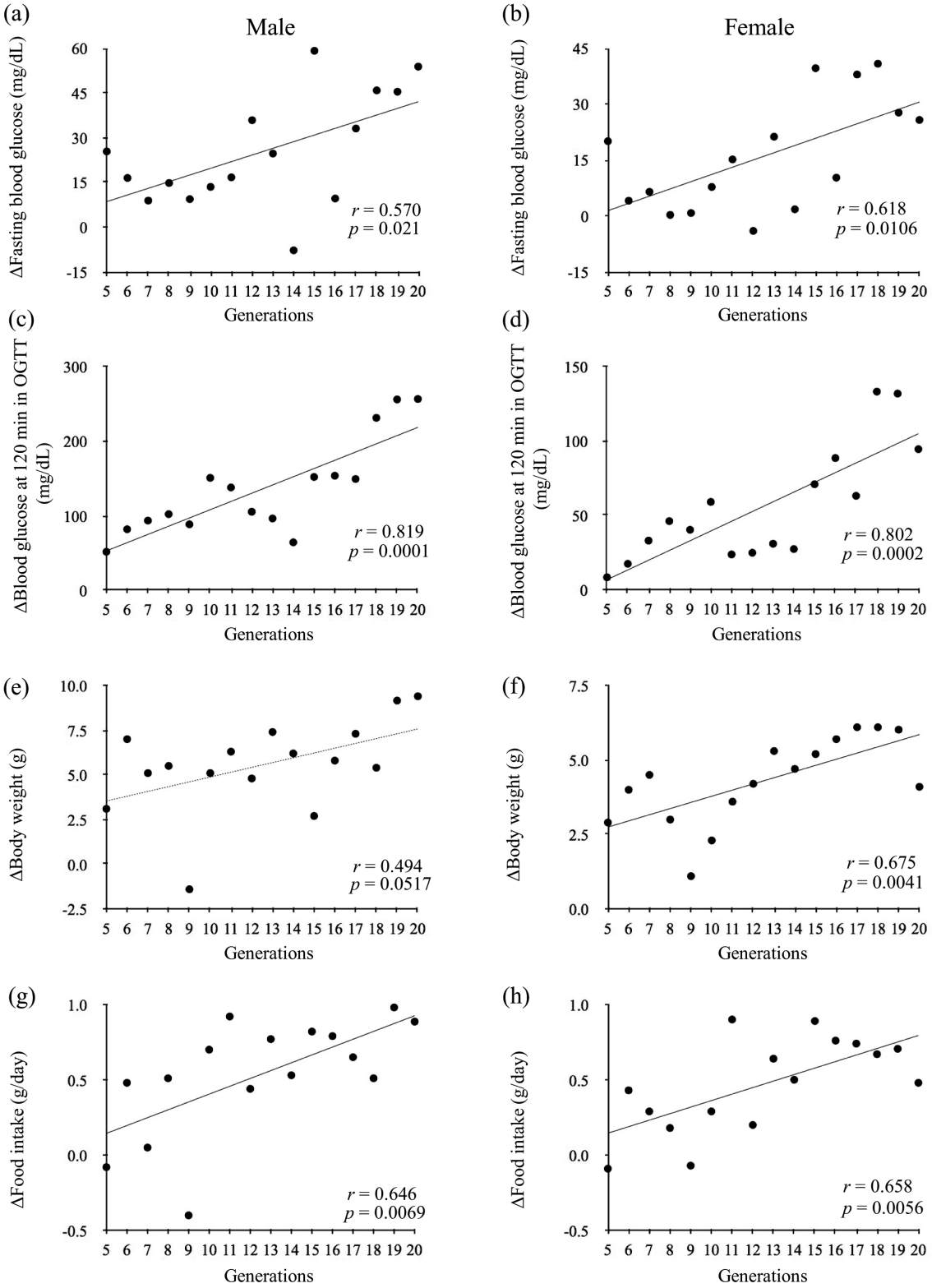

Fig. 2 Intergenerational transition of the differences in metabolic phenotypes.
Each dot indicates the mean value difference between SDG-P and SDG-R (=[SDG-P]–[SDG-R]) of fasting blood glucose (a, b), blood glucose at 120 min in OGTT (c, d), body weight after 5-week HFD feeding (e, f), or HFD intake (g, h) in each generation. Correlation coefficient and *p* value by Pearson correlation analysis are shown in each panel.

Discussion

Transgenerational phenotypic changes have been commonly observed in selective breeding studies. In the establishment of GK rat, Goto *et al.* [1] reported gradual elevation in fasting and post-challenge blood glucose levels over the multiple generations. Similar gradual changes were shown in the selection index traits of NSY mouse [2] and SHR rat [9] during the selective breeding. As a consequence of selective breeding, several independent genetic loci containing genes regulating their metabolic impairments were identified by genetic linkage analyses in the established animal models [10-12]. Hence, the gradual changes in the metabolic phenotypes shown in the present study suggest that the genetic loci associated with the quantitative traits have been selectively enriched in SDG-P and SDG-R.

As the result of selective breeding for different susceptibilities to HFD-induced glucose intolerance, FBG, $BG_{120\ min}$, and BG_{AUC} were all increased with successive generations in SDG-P mice. Unexpectedly, $BG_{120\ min}$ also increased slightly in SDG-R mice, even though we had selected the mice with superior glucose tolerance. Several reports have suggested that parental HFD feeding accelerates HFD-induced glucose intolerance in offspring through epigenetic mechanisms [13-16]. Because both SDG-P and SDG-R mice were fed HFD for selection, the parental HFD feeding may have affected the increasing trend in $BG_{120\ min}$ in both colonies. Nevertheless, the difference in $BG_{120\ min}$ between the 2 colonies became more evident as the generations proceeded, mainly due to the accelerated deterioration of glucose tolerance in SDG-P mice.

Concomitant with the intergenerational transition of glucose tolerance, post-HFD body weight was gradually increased in SDG-P mice in both sexes and slightly decreased in female SDG-R mice; consequently, the difference in post-HFD body weight between the 2 colonies became more evident, especially in the females. HFD-induced body weight gain may induce insulin resistance and promote the development of type 2 diabetes. Indeed, we previously reported that SDG-P mice show inferior insulin sensitivity as compared with SDG-R mice after HFD feeding [8]. The insulin resistance with body weight gain may contribute to the increasing trend in FBG in SDG-P mice. In contrast, the decreasing trend in post-HFD body weight in female SDG-R mice may contribute to improving insulin sensitivity and consequently to lowering blood glucose levels over the repetitive selection.

Of note, SDG-R mice had reduced HFD intake as the generations proceeded, suggesting that SDG-R mice had adapted feeding regulation under HFD to maintain normal glucose tolerance through the selective breeding. Epidemiological data indicate that lower insulin secretion can predict subsequent weight gain; in contrast, higher insulin secretion predicts less food intake and lower weight gain in the Pima population [17, 18]. Thus, although the causal mechanisms have not yet been fully elucidated, superior glucose-induced insulin secretion in SDG-R as compared with SDG-P [8] may contribute to the transgenerational decrease in food intake. In addition, spontaneous central leptin resistance has been proposed as a cause of abnormal feeding behavior in several selective breeding models of metabolic diseases (*e.g.*, GK rat and DIO rat) [5, 19]. Accordingly, different secretion profiles of or different central sensitivities to these anorexigenic factors (insulin and leptin) may be involved in the different feeding behavior between SDG-P and SDG-R mice.

Despite the increasing trend in body weight, HFD intake was not significantly changed in SDG-P mice, suggesting that SDG-P mice had acquired a "thrifty metabolism" through the selective breeding. These results suggest that the phenotypes of energy balance (feeding behavior, energy expenditure, or both) had been concomitantly selected through the selective breeding along with the post-HFD glucose tolerance as a sole selection criterion. Regarding energy expenditure, various traits in voluntary exercise [20, 21] and energy use efficiency [22] have been segregated by selective breeding in rodents. These hereditary traits in feeding behavior and energy expenditure have received much attention in relation to the genetic basis of obesity and obesity-related metabolic disease [23, 24].

As distinct differences have been seen in $BG_{120\ min}$ over generations, we considered that the 2 colonies (SDG-P and SDG-R) were established as phenotypically different lines around the 20th generation. However, since we have performed group breeding with avoiding sister-brother mating (in closed colony since the 5th generation), the 2 colonies were not inbred strains; *i.e.*, certain genetic heterogeneity

is conserved in the 2 colonies. Although the genetic diversity should play pivotal role in establishing polygenic disease model animals by selective breeding, producing inbred strains from the outbred model animals may have potential advantages to identify the culprit genes. Sib-breeding trial is therefore ongoing to establish inbred strains from the 2 lines of mice.

In conclusion, the transgenerational analyses of metabolic phenotypes in SDG-P and SDG-R mice revealed that not only the selection index $BG_{120\ min}$ but also several other traits (FPG, food intake, and body weight) changed gradually to increase the differences between the 2 colonies over the repetitive selection. In addition, the concomitant changes in food intake and body weight with $BG_{120\ min}$ suggest that the energy balance disorders (*i.e.*, hyperphagia and obesity) and glucose metabolism impairment share common etiologies as well characterized in human metabolic syndrome. Taken together, the present results support the usefulness of the selectively bred mouse lines SDG-P and SDG-R for investigating the pathogenesis of multiple metabolic disorders.

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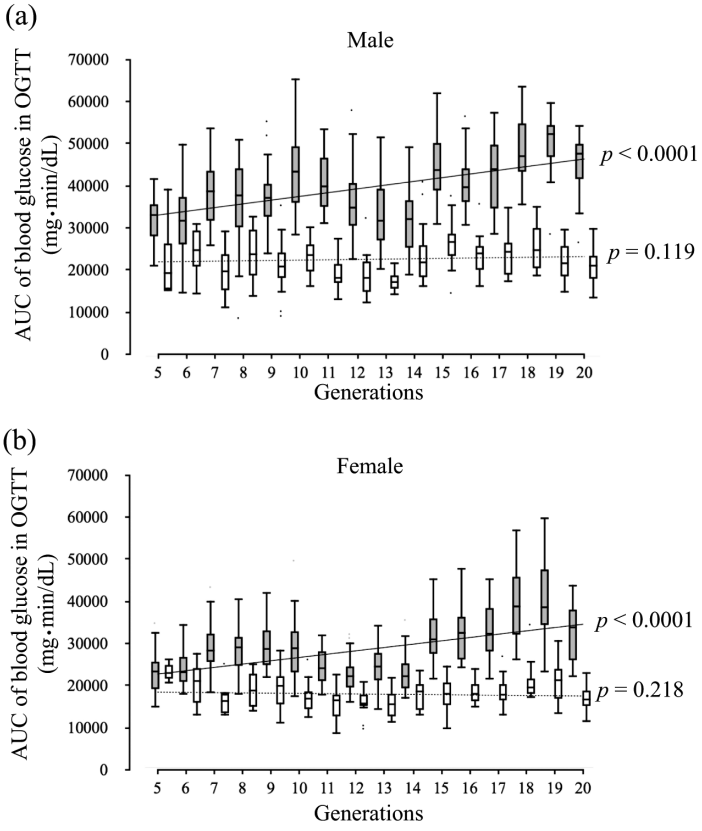
Disclosure

None of the authors have any potential conflicts of interest associated with this research.

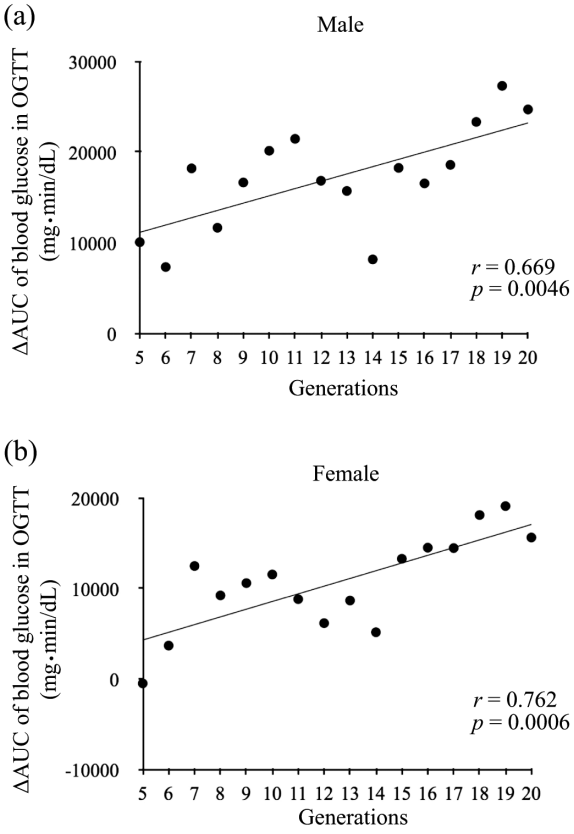
Supplementary Table S1 The total number of mice in each generation and the number of selected pairs for breeding

Generation	SDG-P			SDG-R		
	Female	Male	Selected pairs[1]	Female	Male	Selected pairs[1]
5	41	34	15	7	8	6
6	42	45	8	12	12	5
7	27	31	12	10	13	10
8	19	26	13	15	17	15
9	16	22	16	17	22	10
10	40	29	12	20	23	3
11	21	20	5	20	21	6
12	41	35	10	19	18	5
13	39	43	11	34	18	8
14	42	30	15	26	23	10
15	39	30	15	25	26	12
16	27	27	11	19	15	8
17	41	25	10	20	20	6
18	29	28	10	18	17	7
19	23	13	6 (2:1)[2]	18	23	12
20	15	19	12	17	18	6 (2:1)[2]

[1] Sister-brother mating was avoided. One female was mated with one male unless otherwise specified.
[2] Two females were mated with one male.



Supplementary Fig. S1 Intergenerational distributions of area under the curve (AUC) of blood glucose in OGTT.
AUC of blood glucose in OGTT after 5-week HFD feeding (a, b) in SDG-P (gray boxes) and SDG-R (white boxes). The boxes plot the central rectangular spans from the first quartile to the third quartile (interquartile range; IQR) of each breeding generation. A segment inside the rectangle shows the median. The whiskers display the lowest datum within 1.5 IQR of the lower quartile and the highest datum within 1.5 IQR of the upper quartile. Data not included between the whiskers are plotted as outliers (dots). The *p* values by Jonckheere-Terpstra trend test are shown in each panel.



Supplementary Fig. S2 Intergenerational transition of the differences in area under the curve (AUC) of blood glucose in OGTT.
Each dot indicates the mean value difference between SDG-P and SDG-R (=[SDG-P]–[SDG-R]) of AUC in OGTT after 5-week HFD feeding (a, b) in each generation. Correlation coefficient and *p* value by Pearson correlation analysis are shown in each panel.

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