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**High Fat Diet Load Study in a Natural Obesity-Resistant Animal Model,
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Abstract

Our previous study, demonstrated the obesity-resistant phenomenon in the House musk shrew, *Suncus murinus* (*S. murinus*). In order to go further to explore the mechanism of the phenomenon of natural obesity resistance in *S. murinus*, we focused on the effects of diet on fat accumulation and metabolism. In this study, four-week-old male *S. murinus* were assigned to 1 of 2 experimental groups (n = 6 per group) and fed either a high-fat diet (HFD) or a normal trout diet (NTD) for 24 weeks. Body weight, food ingestion, visceral fat distribution, blood biochemistry and fecal lipids were monitored and analyzed in the HFD and NTD groups. It was found no differences in the average final body weight or body fat change between the two groups. Although animals fed HFD had similar serum triglyceride concentration to animals fed NTD, they had significantly higher phospholipid and total cholesterol concentrations than the NTD group. Furthermore, fecal lipid levels in the HFD group was significantly higher than those in the NTD group, suggesting that diet composition or energy density of the diet affects blood biochemistry. Although NTD-fed *S. murinus* had higher food ingestion than HFD-fed *S. murinus*, caloric intake remained almost the same. We speculated *S. murinus* may suppress obesity and control fat accumulation by controlling calorie intake. These results suggest that the obesity characteristics of *S. murinus* are not primarily due to dietary factors but to other mechanisms of regulation.

Keywords: High fat diet; Mesenteric fat; Obesity-resistant; *Suncus murinus*

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Introduction

Obesity is defined as excessive fat accumulation that leads to higher risk of associated co-morbidities including diabetes, cancer and cardiovascular disorders [1]. Worldwide, the prevalence of obesity has nearly tripled since 1975, with 39% of adults worldwide being overweight and 13% being obese [2]. Estimations from the noncommunicable diseases collaboration reported that if these trends continue, then by 2025, global obesity prevalence will reach 18% in men and surpass 21% in women, and severe obesity will surpass 6% in men and 9% in women [3]. Thus, more attention should be paid to the significant and interrelated public health issues related to obesity. What has caused this rapid increase in obesity within less than one generation? The past century has seen marked changes in human lifestyle, ranging from new dietary patterns to improved hygiene and altered sleep-wake cycles [4]. The high prevalence of obesity may be partially attributed to increasing consumption of hypercaloric foods and sedentary lifestyle [2, 5]. To develop new therapeutic treatment or dietary interventions, a study model for obesity should be developed. A number of animal models have been developed [6-9]; however, there are no suitable resistant to human-type obesity animal models. The house musk shrew, *Suncus murinus*, is a small mammal that belongs to the order Insectivora, family Soricidae and genus *Suncus*, and lives in houses and grassy areas near human habitations or in cattle pens. *S. murinus* eat insects and worms, is nocturnal [10-12], and widely distributed throughout Asia and East Africa [13, 14]. In Japan, presumably native populations are distributed in the Nansei Islands [the Amami, Okinawa, Miyako and Yaeyama Islands]. *S. murinus* was established from wild animals as a

laboratory model system in Japan, and strains differ in body weight [15]. Because *S. murinus* has different characteristics to mice and rats, they have been widely used for physiological and morphological experiments [16]. *S. murinus* are reported to possess features closer to those of primates than rodents on the basis of, for example, mitochondrial DNA sequences [17, 18]. This species has been used in various fields of science due to its unique characteristics compared with laboratory rodents, including use as models of emesis, and utilization for studies of cold intolerance and brown adipose tissue [19-21]. Further more, *S. murinus* has a visceral system that is very similar to that of humans and is a useful model of human physiology and pathophysiology [10-12, 22-29]. In our previous study, we investigated the obesity-resistant phenomenon in *S. murinus* [26]. Body weight changed little during growth (aging) from 2 months up to 12 months of age, and less visceral fat accumulated; in particular, mesenteric fat accumulation did not occur until old age in this species. Thus, *S. murinus* may be a suitable model for investigating obesity and metabolic syndrome, particularly the mechanism of obesity resistance [26]. In the present study, in order to go further to explore the mechanism of the phenomenon of natural obesity resistance in *S. murinus*, we focused on the effects of diet on fat accumulation and metabolism. Body weight, food ingestion, visceral fat distribution, blood biochemistry and fecal lipids were monitored and analyzed in *S. murinus* fed on high-fat and normal trout diets.

Materials and Methods

House musk shrew, *Suncus murinus*

The experiment was performed using *S. murinus* (male, n = 12, 4-week-old) obtained from a closed breeding colony, J1c: KAT-c, at

our laboratory [22, 25]. All animals were housed in polycarbonate cages in a room maintained at 28 ± 2 C° with relative humidity of 50 ± 5 % in the Functional Morphology Laboratory, Department of Frontier Health Sciences, Tokyo Metropolitan University, Japan. The room was automatically lit between 9:00 h and 21:00 h. Pellets and water were supplied *ad libitum*.

Experimental design and procedure

All experimental procedures were approved by the institutional Animal care utilization committee of Tokyo Metropolitan University and performed in accordance with institution guidelines (No. A31-34). The twelve male *S. murinus* were randomly divided into two

groups and fed either a high-fat diet (HFD group, n = 6, weight 55.30 ± 5.94 g), Purina One (Nestle Inc., St. Louis, USA), which is tender selects blend with real chicken adult dry cat food for kitty cats; or a normal trout diet (NTD group, equivalent to a general diet chow, n = 6, weight 63.92 ± 8.03 g), which is a specific and natural food of this insectivorous species (Oriental Yeast Co., Ltd. Bioindustry Division, Chiba, Japan). The contents and metabolizable energy contents of the diets are listed in Table 1. In addition, the animals from the same litter were separated into the two groups, and there was no significant difference in mean body weight between the two groups in the initial stage of the experiment ($p > 0.05$).

Table 1: Formulation of the high-fat diet and normal trout diet

	Protein %	Fat %	Fiber %	Ash %	Carbohydrate %	H ₂ O %	Others %	Kcal / 100g
Trout diet	45	4	3	15	26.2	6.8		357
Purina one	40	18	2.5	9	14.6	12	3.9*	440

* Others: 1.0% calcium, 0.8% phosphorus, 0.1% magnesium, 0.18% taurine, 1.8% linoleic acid, and Vitamin A, E and D

Determination of body weight and food consumption

To characterize weight gain and energy ingestion, body weight and food consumption were monitored weekly.

Determination of serum fat parameters

After 24 weeks, the animals were sacrificed by anesthesia. Blood was collected and centrifuged at 3000 rpm for 10 min at 4 C°, and serum chemistry for total cholesterol (TCho) (Co. 439-17501; Wako Inc., OSA, Japan), triglycerides (TG) (Co. 432-40201; Wako Inc., OSA, Japan) and phospholipids (PL) (Co. 433-36201; Wako Inc., OSA, Japan) was analyzed using an automatic chemistry analyzer according to the manufacturer's protocol.

Measurement of the quantity of lipid in feces

According to the manufacturer's protocol, fresh feces were collected from the cages and oven-dried for 12 hours at 65 C° to evaporate the water. 0.5 g of the dried feces were extracted using 10 ml of concentrated hydrochloric acid (12 mol/dm³) for 30 min at a constant temperature of 37 C° in a water bath. Then, 50 ml ether was added after cooling, after shock to be stratified, and 25 ml of supernatant was collected into another test tube. After allowing the ether to evaporate completely at room temperature, the lipid extract was weighed.

Statistical analysis

The data are displayed as mean and standard deviation [mean \pm SD]. Differences between

the groups were analyzed with the Students *t* test and a *p* value < 0.05 was considered significant (GraphPad Prism 5.1, GraphPad Software, La Jolla, CA, USA).

Results

Body weight

The changes in body weight in the two *S. murinus* groups during the experiment period

are shown in figure 1. At 24 weeks of the experiment, the HFD and the NTD groups had a mean weight of 86.02 ± 6.99 g and 83.57 ± 8.91 g, respectively, no significant difference between HFD group and NTD group ($p = 0.6085$). During the study period, the body weight gained in HFD group and NTD group were 29.18 ± 11.27 g and 21.33 ± 4.64 g, respectively, it was not significantly higher in the NTD group ($p = 0.1609$).

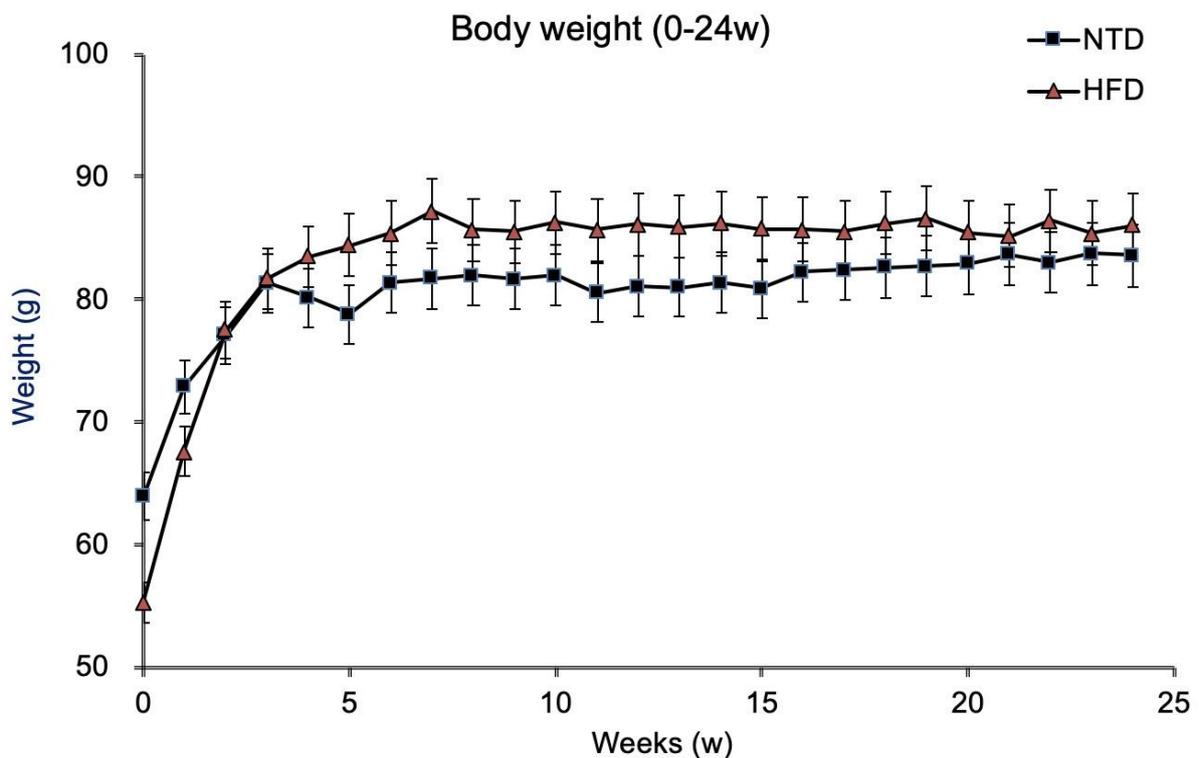


Figure 1: Body weights of *S. murinus* over the 24-week experimental period.

Food ingestion

The changes in food ingestion in the two *S. murinus* groups were shown in figure 2. The quantity of food ingested was significantly lower in the HFD group than the NTD group from the beginning of the study (5.73 ± 0.68 vs 4.44 ± 0.37 g/day, $p < 0.001$). However, the average energy ingested per day during the entire study was not significantly higher in the HFD group than in the NTD group (19.55 ± 1.64 vs 20.47 ± 2.44 Kcal/day, $p = 0.1012$).

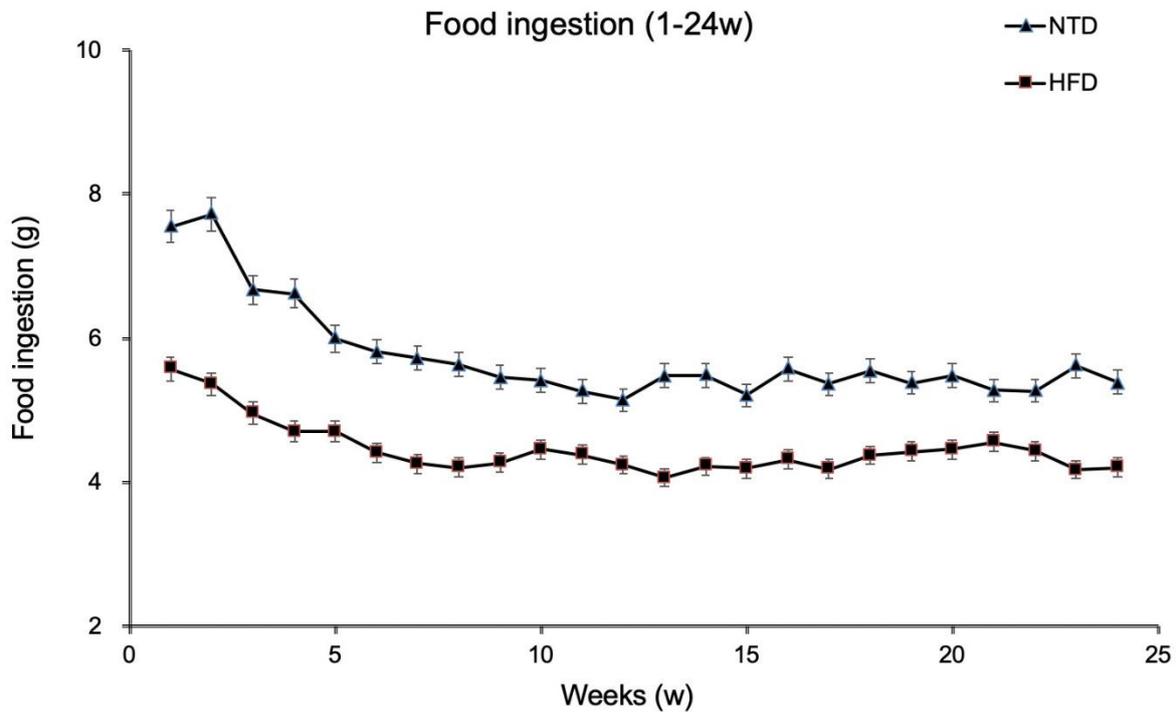


Figure 2: Food ingestion of *S. murinus* over the 24-week experimental period. There was a significant difference between the groups ($p < 0.001$).

Body fat

To examine the effect of HFD supplementation on body fat distribution, we performed laparotomy after completion of the study and evaluated changes in body fat. The distribution and quantity of subcutaneous white fat, epididymal fat, mesenteric fat (Figure 3) and retroperitoneal fat (data not shown) were not markedly different between the two groups.

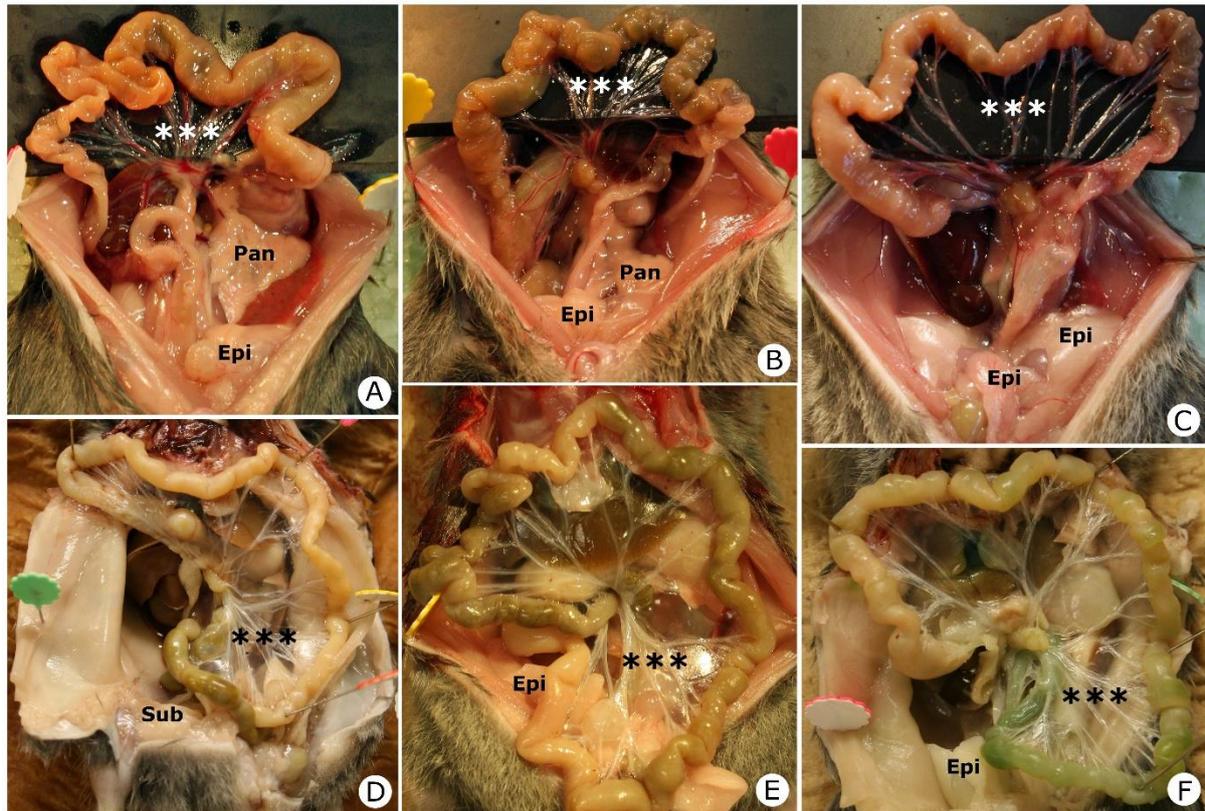


Figure 3: Representative body fat pictures of the HFD group (A, B and C) and NTD group (D, E and F) for each experiment after 24 weeks of treatment.***, mesenterium; Epi, epididymal fat pad; Pan, pancreas; Sub, subcutaneous fat.

Blood biochemistry

The serum phospholipid and total cholesterol concentrations were significantly increased in the HFD group compared with the NTD group ($p = 0.003859$ and $p < 0.001$, respectively); however, the concentration of triglyceride was similar between two groups ($p = 0.0803$) (Figure 4).

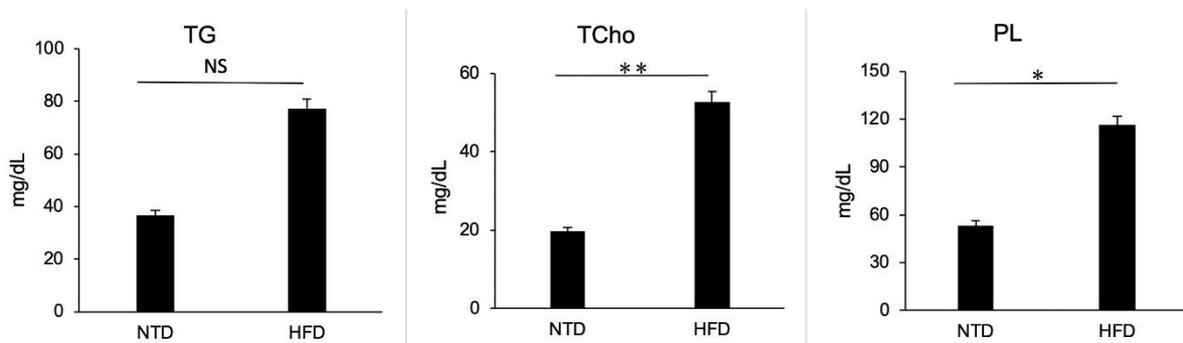


Figure 4: Serum triglyceride (TG), phospholipid (PL) and total cholesterol concentrations (TCho) in *S. murinus* in the HFD and NTD groups after 24 weeks of treatment. Between the two groups, there

were significant differences in PL ($*p < 0.05$) and Tcho ($**p < 0.001$), and no significant difference in TG ($p > 0.05$).

Fecal lipids

The fecal excreted by *S. murinus* was displayed as semisolid state in the HFD group, while it was presented as solid one in the NTD group. The fecal lipid level in the HFD group was significantly higher than that in the NTD group (0.0154 ± 0.0006 vs 0.0106 ± 0.0033 , $p = 0.0287$) (Figure 5).

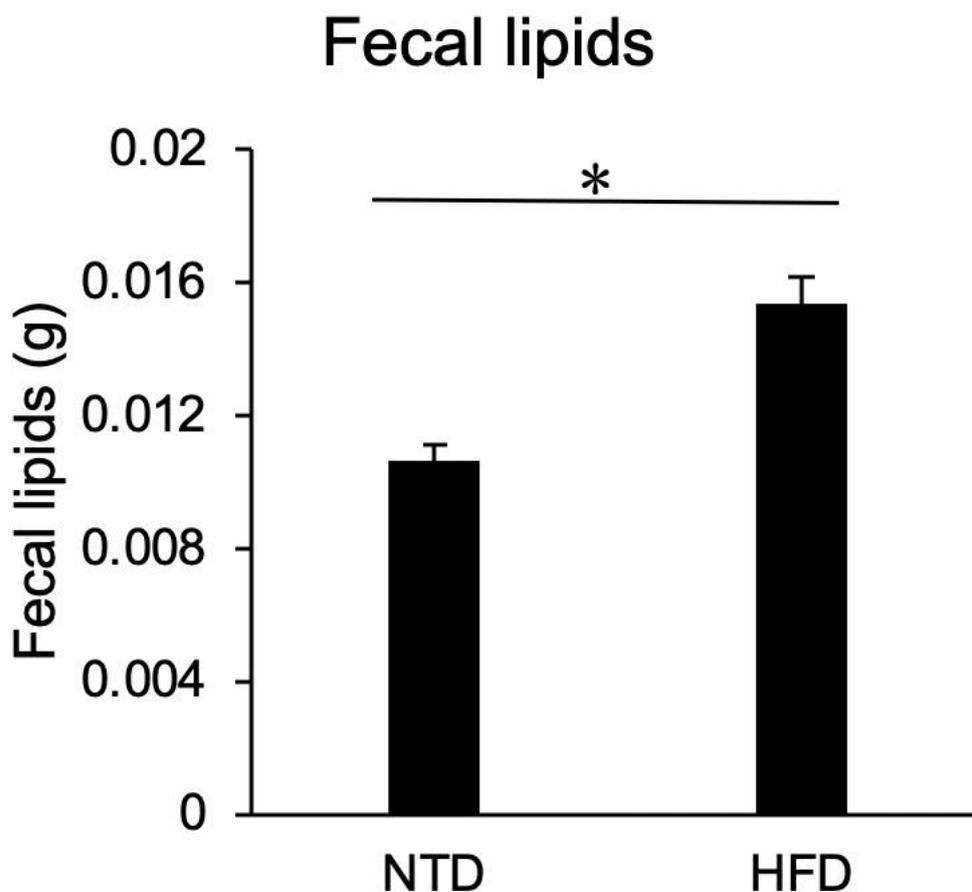


Figure 5: Fecal lipids levels in *S. murinus* after feeding in the HFD group and NTD group. There was a significant difference between two groups ($*p < 0.05$).

Discussion

In this study, we examined the mechanism of obesity-resistance in *S. murinus*, a novel and naturally obesity-resistant animal model [26]. Body weight, food ingestion, visceral fat distribution, blood biochemistry and fecal lipids were examined and analyzed in the animals fed high-fat diet or normal trout diet. We found no significant changes in body

weight or fat distribution. However, changes in blood serum fat were significantly different between the two groups. Interestingly, food intake was significantly higher in the NTD group and fecal lipids were significantly higher in the HFD group.

In our preliminary experiment, the high-fat diets HFD45 and HFD32 that are commonly used for mice or rats were first selected, but the experiment could not proceed because *S.*

murinus refused to eat them. Therefore, the diet was changed to Purina One with a fat content of 18.0%, which the animals consumed. Purina One has more than four times the fat content of the conventional NTD of *S. murinus* (4.0%). Calorie levels for the HFD and NTD are 440 Kcal/100g vs 357 Kcal/100g, respectively, and HFD is 23% higher than NTD. Although our study did not use the conventional HFD30-50 as a high-fat diet, we don't think this will affect the study of the effects of higher fat, higher calorie diets on *S. murinus* fat metabolism and deposition.

The present study showed that HFD feeding for 24 weeks in *S. murinus* did not significantly increase body weight, subcutaneous white adipose or visceral fat compared with NTD-fed *S. murinus*. In particular, mesenteric fat was not observed in the HFD group. Several different mechanisms have been speculated in our previous study [26], including growth environment, structure of the gastrointestinal tract, diet composition and existence or differentiation of mesenteric adipose stem cells of *S. murinus*, but the actual mechanism was unclear. In the present study, we found that food intake was significantly higher in the NTD group than the HFD group ($p < 0.001$) from the beginning of the experiment. However, interestingly, daily calorie intake was not significantly different between the two groups. Thus, we speculate that *S. murinus* suppressed obesity and controlled fat accumulation by controlling calorie intake. Different theories have been suggested such as absorbed calories may provide feedback on food intake. Another possibility is that the autonomic nervous system controls the flow of energy through regulation of storage and mobilization of nutrients as well as by regulating the flow of energy through valve uncoupled, which does not generate ATP. Alternatively, the sympathetic nervous system may have an important role in the regulation of metabolism and the long-term maintenance of energy balance [30].

We also evaluated the baseline pathological data of *S. murinus* fed HFD including serum fat and fecal fat. The results showed that

phospholipids and total cholesterol were significantly increased ($p < 0.05$ and $p < 0.001$, respectively), while triglyceride concentration did not significantly change in the HFD group. Triglyceride fractions in blood are considered to be influenced by the type and amount of fat consumed during recent meals [31]. Since triglyceride levels did not significantly change, we speculate that the amount of fat consumed was almost the same between the two groups. Serum phospholipids mirror the dietary intake of recent months and, roughly, the composition of body fat [32]. Numerous studies have shown that cholesterol intake is closely related to dyslipidemia. This is because the intake of high fat can increase the secretion of bile acids in the intestine, and this increase promotes the absorption of cholesterol in the daily diet and a subsequent increase in cholesterol in the blood [33]. Our results suggest that a high-fat diet increases cholesterol levels in *S. murinus*. The most likely explanation for the obesity-resistance in *S. murinus* may be that this species possesses specific metabolic effect of differing proportions of fat and carbohydrate in the diet.

In this study, the fecal fat extracted in the HFD group was higher significantly than in the NTD group ($p < 0.05$). This increase in fecal lipid contents may be due to the changes in cholesterol and triglyceride absorption in the intestines. It was suggested that the undigested protein influences gut absorptive function by either blocking the absorption or sequestering of cholesterol, thereby enhancing the excretion of cholesterol via feces [34]. Nevertheless, the increased excretion of fecal lipids partly explains the obesity resistance in HFD-fed *S. murinus*. Therefore, we suggest that increases in fecal fat excretion may be due to a decrease in fat absorption in *S. murinus*. Thus, we speculated that the HFD group had malabsorption based on semisolid excrement compared with that in the NTD group. In conclusion, based on our and previous findings, a high-fat diet does not induce obesity in *S. murinus*. This may be because *S. murinus* fed HFD may control visceral and mesenteric fat accumulation by controlling calorie intake and excreting excess fat through their feces.

However, the mechanism of fat-storage homeostasis in *S. murinus* will be an important subject in our continuing investigation.

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Author contributions

Zhang M, Ren K and Yi SQ designed the research; Zhang M, Dai Y and Wang Z performed all experiments, collected data and analyzed the data; Terayama H, Sasaki H and Yi SQ performed the statistical analysis; Zhang M wrote the manuscript; Chen J, Yamamoto Y and Yi SQ reviewed the manuscript; all authors approved the final version of the manuscript.

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