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# Sufficient intake of high-fat food attenuates stress-induced social avoidance behavior

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## Abstract

**Aims:** Psychosocial stress is a form of mental stress associated with human relationships that underlies the pathogenesis of mental disorders such as depression. Previous studies have suggested that intake of energy-dense foods, also known as "palatable foods," can relieve psychosocial stress. However, it remains unclear whether the volume of palatable food affects abnormal behavior induced by psychosocial stress. In the present study, we aimed to determine whether levels of high-fat food intake significantly influence psychosocial stress using the social-defeat stress (SDS) paradigm.

**Main methods:** Mice subjected to SDS ate either a high-fat or normal chow diet for 10 days. Behavioral tests were conducted following the completion of the SDS paradigm. The hypothalamus, liver, and blood were examined post-mortem.

**Key findings:** Mice with sufficient intake of high-fat chow immediately following exposure to SDS did not exhibit social avoidance behavior, suggesting that a high-fat diet may improve social behavior. However, inadequate intake of high-fat food, which did not alter cholesterol metabolism or hypothalamic-pituitary-adrenal axis activity, was not associated with such benefits, instead increased anxiety-like behavior.

**Significance:** The results of the present study demonstrate that eating a high-fat diet may attenuate stress, but that this benefit disappears with insufficient intake of high-fat foods. The benefits of a high-fat diet under SDS may be related to cholesterol metabolism in the liver.

**Keywords**: social defeat stress, social avoidance, high-fat diet, HPA axis, depression, anxiety

## 1. Introduction

Psychosocial stress is a form of mental stress associated with human relationships that underlies the pathogenesis of mental disorders such as depression [1,2]. Depression leads to serious social and educational impairments and is also a major risk factor for suicide [3,4].

Some studies have revealed that undue stress leads to alterations in food preferences [5-8], and that humans under stress prefer to eat more calorie-dense foods [8-10]. In addition, rodents exposed to various types of stress exhibit increased intake of energy-dense foods, also known as "palatable foods" [11,12]. Researchers have suggested that palatable food consumption represents one strategy for attenuating negative emotions (e.g., anxiety) induced by various stressors [11,13,14]. In addition, stress is known to increase the preference for high-fat foods in certain individuals [12,15]. Some studies have further reported that high-fat diets reduce both autonomic and hypothalamic-pituitary-adrenal (HPA) axis responses to repeated stressors in rodents [11,16-20], suggesting that high-fat diets affect the stress response modulation. In addition, recent human studies have revealed that ketogenic diets (high fat, low protein, low carbohydrate) may aid in the treatment of mood disorders [21]. Animal studies have supported this notion, as rats subjected to ketogenic diets spend less time immobile during the forced-swim test, indicative of improvements in depression-like behavior [22].

Obesity is a major risk for metabolic disorders such as diabetes [23,24]. Recent reports have revealed that long-term ingestion of a high-fat diet induces abnormal behavior and increases obesity risk [25-27]. Thus, although high-fat diets may mitigate psychosocial stress in humans, obesity due to over-consumption of high-fat foods may induce behavioral disorders through an unidentified metabolic system. It is therefore necessary to determine the fat intake level appropriate for reducing psychosocial stress without increasing the risk of obesity-related complications.

In the present study, we evaluated the effect of a high-fat diet on psychosocial stress using the social-defeat stress (SDS) model, which is among the major stress paradigms used to induce the equivalent of human psychosocial stress in rodents [28]. Mice subjected to SDS based on the "resident-intruder paradigm" exhibit alterations in behavior, such as an increase in social avoidance [28,29], which has been shown to improve with the administration of anti-depressants. Therefore, SDS-induced social avoidance is used as a measure of depressive-like behavior and/or sociality.

Previous studies have reported that voluntary exercise for 2 hours immediately after stress exposure reduces SDS-induced social avoidance behavior [30]. In the absence of

hunger, humans tend to choose more energy-dense foods during acute stress than under rest conditions [31]. Further, high-fat diet intake affects hepatic lipid metabolism, while chronic stress disrupts the regulation of lipid synthesis in the liver [32]. Therefore, we hypothesized that a restricted high-fat diet that did not increase the obesity risk might attenuate psychosocial stress without inducing metabolic disorders. Using the SDS paradigm, we investigated whether the fat intake level influences improvements in psychosocial stress and such improvements are associated with alterations in HPA axis activity and hepatic lipid metabolism.

## 2. Materials and methods

### 2.1 Animals and group design

Eight-week-old male C57BL/6 mice (Japan SLC, Shizuoka, Japan) were used in the present study. Aggressor mice consisted of retired male ICR mice (Japan SLC, Shizuoka, Japan) (n = 24; these mice were not sacrificed.). All mice were individually housed and maintained on a 12-hour light/dark cycle (lights on at 9:00 am), with food and water available *ad libitum* for 2 weeks prior to experimental procedures. Experimental mice were separated into six groups for each experiment (feeding schedule is pictured in Fig. 1A). Experiment 1 included: (1) 22 h of *ad libitum* normal chow diet and no SDS exposure (N-ND; n = 9); (2) *ad libitum* normal chow diet and SDS exposure (S-ND; n = 9); (3) 22 h of *ad libitum* high-fat diet and no SDS exposure (N-HFD-ad; n = 6); (4) *ad libitum* high-fat diet and SDS exposure (S-HFD-ad; n = 6); (5) 22 h of *ad libitum* normal chow diet with 2 h of high-fat diet and no SDS exposure (N-HFD-2h; n = 6); (6) 22 h of *ad libitum* normal chow diet with 2 h of high-fat diet after SDS exposure (S-HFD-2h; n = 6). Experiment 2 included: (1) N-ND (n = 7); (2) S-ND (n = 6); (3) N-HFD-2h (n = 7); (4) S-HFD-2h; (5) 22 h of *ad libitum* normal chow diet with 0.75 g of high-fat diet, which represented half the amount eaten by the HFD 2h group after SDS exposure and no SDS exposure (N-HFD-half; n = 8); (6) 22 h of *ad libitum* normal chow diet with 0.75 g of high-fat diet, which represented half the amount eaten by the HFD 2h group after SDS exposure (S-HFD-half; n = 6). Experimental animals were randomly assigned to the aforementioned groups, which exhibited no significant differences in body weight [Experiment 1: N-ND, 23.5 ± 0.4; S-ND, 23.1 ± 0.4; N-HFD-ad, 22.8 ± 0.6; S-HFD-ad, 22.5 ± 0.4; N-HFD-2h, 23.3 ± 0.4; S-HFD-2h, 22.7 ± 0.2; Experiment 2: N-ND, 24.6 ± 0.5; S-ND, 27.3 ± 0.5; N-HFD-2h, 24.1 ± 0.3; S-HFD-2h, 26.8 ± 0.6; N-HFD-half, 24.2 ± 0.4; S-HFD-half, 25.1 ± 0.5]. The present study was approved by the Animal Study Committee of Tokushima University (Toku-13109) and conducted in accordance with the Guidelines for the Care and Use of Animals of the Council of the Physiological Society of Japan.

### 2.2 SDS and high-fat diet paradigms

The SDS paradigm was developed based on previously described methods, with slight modifications [28]. In this paradigm, experimental mice were placed into an aggressor's home cage for 2.5 min (or until the aggressor had performed five attacks) for 10 days. After the physical interaction, experimental mice were returned to their home cages, which lay side-by-side with an aggressor's home cage, following which the S-HFD-ad, S-HFD-2h, and S-HFD-half groups were given high-fat chow (Fig. 1B, Fig. 2).

Behavioral tests were conducted following the completion of the SDS paradigm. Mice in the high-fat groups were individually housed in cages with high-fat chow (containing 5.2 kcal/g, with 60% of the calories from fat, 20% from protein, and 20% from carbohydrates; D12492, Research Diets, NJ, USA) for 24 h (HFD-ad groups) or 2 h (HFD-2h and HFD-half groups) during the experimental paradigm, and were habituated for 3 days prior to experimental procedures. Feeding schedules are pictured in Fig. 1B.

### 2.3 Open-field (OF) test

To assess the effect of SDS on anxiety-like behavior in Experiments 1 and 2, we evaluated the locomotor activity of experimental mice in an open-field chamber that consisted of an acrylic box (50 cm × 50 cm × 30 cm). Mouse behavior, total distance traveled, and time spent in the central area (25% of the box) were monitored for 10 min and analyzed using the Image OF program (O'Hara, Tokyo, Japan) derived from ImageJ 1.34s (National Institutes of Health, Bethesda, MD, USA).

### 2.4 Social interaction test

To assess social behavior, experimental mice were evaluated during a social interaction (SI) test in the open-field chamber in Experiments 1 and 2, in accordance with previously described methods [30]. The chamber was separated into three zones: the interaction zone (25% of the central area), corner zone (9 cm × 9 cm; four positions in the corner areas), and others. The SI test was performed two times, and mouse behavior was monitored for 2.5 min in each session. During the first test, an empty gauze cage was placed in the central area. The experimental animal was then placed back in its home cage for 1 min. During the second test, the same gauze cage was placed in the central area, although it now contained the aggressor mouse. We then measured the total distance traveled and time spent in the interaction zone (25%). Mouse behavior was also then analyzed using the Image OF program (O'Hara) derived from ImageJ program. Time spent in the corner zone was analyzed based on video recordings.

### 2.5 Light–dark test

Anxiety-like behavior was assessed using a light–dark box comprised of two 20 cm × 20 cm × 25 cm compartments in Experiment 1. The light compartment consisted of a white floor, walls, and a lid, and was illuminated using a light-emitting diode, while the dark compartment consisted of a black floor, walls, and a lid. The two chambers were completely enclosed except for a small opening (3 cm × 5 cm) to allow movement of the mice from the dark compartment to the light compartment. The experimental

mice were placed into the dark compartment without opening the door. After 5 seconds, the door was opened, and behavioral recording began. The total distance traveled and the amount of time spent in each compartment were also recorded for 5 min using the Image LD program (O'Hara), which is also based on the Image J program. A greater amount of time spent in the dark chamber has been established as an index of anxiety-like behavior [22].

### 2.6 Tail suspension test

The tail suspension **(**TS) test was used to assess depressive-like behavior in experimental mice in Experiment 1. During the test, the tip of the mouse's tail was fixed with adhesive tape to a wire dangling from the ceiling of the cage. The percentage of time that mice spent immobile was measured for 5 min. Decreases in the time spent immobile are considered to indicate decreases in depressive-like behavior [16]. Data were recorded using the Image FST program (O'Hara), which is also based on Image J.

### 2.7 Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Sampling was performed 24 h after final behavioral tests. The experimental mice were decapitated, the whole brain was removed, and the dissected hypothalamus (between 0.0 and 2.0 mm posterior to the bregma) was used for real-time RT-PCR. The hypothalamic region was dissected from 1-mm-thick coronal sections of the fresh brain with the brain orientated for sectioning according to the mouse stereotaxic atlas [33]. RNA was prepared from the liver and hypothalamus of mice using a commercially available isolation protocol (RNAiso Plus; Takara Bio, Shiga, Japan). A Gene Amp RNA polymerase chain reaction (PCR) kit was used to generate cDNA (Applied Biosystems, Foster, CA). We used pre-designed, gene-specific SYBR Green probes and primer sets to assess the expression of the following genes: CPT1a (Cpt1a), PPARα (Ppara), PGC1α (Ppargc1a), SREBP1 (Srebf1), FAS (Fasn), Cyp7a1 (Cyp7a1), CRH (Crh), and β-actin (Actb). Primers for the genes are shown in Table 1. The real-time RT-PCR reaction was performed using an Applied Biosystems 7900HT real-time RT-PCR system and SYBR Green PCR Master Mix (Roche Diagnostics, Indianapolis, USA), in accordance with the manufacturer's instructions. Stress might affect the expression of housekeeping genes [34]. In the levels of β-actin mRNA expression, we did not observe a significant effect of SDS (liver: $F_{1,30}$ =0.497, p = 0.4861; hypothalamus: $F_{1,30}$ =2.866, p = 0.1008). Therefore, for endogenous quantity control, each gene expression value was normalized to each level of β-actin mRNA expression.

### 2.8 Plasma corticosterone and cholesterol levels

Plasma corticosterone levels were measured using an ELISA kit (YK240, Yanaihara Institute Inc., Shizuoka, Japan), in accordance with the manufacturer's protocol. The Plasma cholesterol concentration in the test solution was analyzed using LabAssayTM Cholesterol (Wako Pure Chemical Industries, Osaka, Japan). Mice were sacrificed via decapitation, and their trunk blood was collected in tubes. Blood samples were centrifuged at 9,000 rpm for 15 min and stored at –30 °C.

### 2.9 Statistical analyses

The individual assays were performed in single. Values are expressed as the mean ± standard error (SE). After verification of homogeneity of variance and normal distribution of data, Two-way analysis of variance analysis of variance (ANOVA) was performed using SDS and diet as factors. If a statistically significant effect was observed, post hoc analysis (Bonferroni) was performed to detect differences between groups. The level of statistical significance was set at $p < 0.05$.

## 3. Results

### 3.1 Restricted high-fat diet (2 h after SDS) did not induce obesity

We first investigated whether a high-fat diet affect the body weight. One group of mice was allowed *ad libitum* access to a high-fat diet (HFD-ad), while the other group was allowed access for only 2 h after SDS (HFD-2h) (Fig. 1A). Total calorie intake during the SDS paradigm for both groups is shown in Fig. 3A and Table 2.

Two-way ANOVA revealed that the high-fat diet was associated with significant increases in body weight (Day 6: $F_{2,30} = 59.82$, $p < 0.001$; Day 11: $F_{2,30} = 38.67$, $p < 0.001$), although SDS was not (Day 6: $F_{1,30} = 0.8046$, $p = 0.376$; Day 11: $F_{1,30} = 0.6069$, $p = 0.442$) (Fig. 3B). Both the N-HFD-ad and S-HFD-ad groups exhibited significant increases in body weight, when compared with the ND groups on Day 6. Despite the ingestion of high-fat chow, as we expected, neither the N-HFD-2h nor the S-HFD-2h group exhibited significant increases in body weight when compared with the ND groups on Days 6 and 11.

We then examined changes in the weight of epididymal white adipose tissue (eWAT). Two-way ANOVA revealed significant main effects of both SDS ($F_{1,30} = 5.708$, $p = 0.023$) and diet ($F_{2,30} = 53.49$, $p < 0.001$), although we observed no interaction between SDS and diet (Fig. 3C). SDS induced decreases in the weight of eWAT. Both HFD-ad groups exhibited significant increases in eWAT when compared with the ND groups, although the N-HFD-2h and the S-HFD-2h groups exhibited no increases in eWAT.

### 3.2 Ingestion of a high-fat diet attenuated SDS-induced social avoidance

We next investigated whether a high-fat diet can attenuate SDS-induced social avoidance behavior. The behavioral results of Experiment 1 are depicted in Fig. 4. The SI test was performed to assess the social preference of experimental mice (Fig. 4A, B). Two-way ANOVA revealed significant interaction effects of SDS and diet on the time spent in the interaction zone ($F_{2,30} = 3.963$, $p = 0.028$) and corner zones ($F_{2,30} = 7.765$, $p = 0.0015$). Neither the S-HFD-ad nor the S-HFD-2h group exhibited decreases in the time spent in the interaction zone, although the S-ND group exhibited a significant decrease relative to the N-ND group. The S-ND group also spent more time in the corner zone, although this difference was not observed in the S-HFD-ad or S-HFD-2h groups.

The OF test and light–dark test were used to assess anxiety-like behavior (Fig. 4C-F). During the OF test, there were no significant differences in the time spent in the center areas of the field among the groups (SDS: $F_{1,30} = 0.675$, $p = 0.4178$; diet: $F_{2,30} =$

1.440, $p = 0.2528$), although SDS exposure decreased the total distance traveled during the OF test ($F_{1,30}$ =9.775, p = 0.0039) (Fig. 4C, D). During the light–dark test, there were no significant differences in the time spent in each compartment (SDS: $F_{1,30}$ =0.0654, p = 0.800; diet: $F_{2,30}$ = 0.6158, $p = 0.5469$) or distance traveled (SDS: $F_{1,30}$ =1.933, p = 0.1746; diet: $F_{2,30}$ = 0.2701, $p = 0.7651$) among the groups (Fig. 4E, F).

We also utilized the TS test to assess depressive-like behavior. However, no significant differences in immobility time were observed among the experimental groups (SDS: $F_{1,30}$ = 0.872, p = 0.3578; diet: $F_{2,30}$ = 0.701, $p = 0.5041$) (Fig. 4G).

Taken together, our findings indicate that exposure to our SDS paradigm induced social avoidance without increasing anxiety- or depressive-like behavior.

### 3.3 Inadequate supply of high-fat food negates the effect of improved social activity and induces anxiety-like behavior

To further assess the effect of a high-fat diet on social behavior, we examined whether a more restrictive high-fat diet than HFD-2h could attenuate SDS-induced social avoidance behavior. Mice in the S-HFD-half group were exposed to SDS and an amount of high-fat chow equal to half that eaten in the HFD-2h group (Fig. 2). We observed no significant difference in energy intake between the N-ND and S-HFD-half groups (Fig. 5A, Table 3). On Day 6, there was no significant difference in body weight gain among the groups (SDS: $F_{1,34}$ = 0.0387, p = 0.8453; diet: $F_{2,34}$ = 0.352, $p = 0.7059$). However, body weight gain was significantly increased in the N-HFD-2h and N-HFD-half groups on Day 11, when compared with that in the N-ND group (SDS: $F_{1,34}$ = 9.00, p = 0.005; diet: $F_{2,34}$ = 6.589, $p = 0.004$) (Fig. 5B). Two-way ANOVA revealed that SDS resulted in significant decreases in eWAT ($F_{1,34}$ = 52.49, $p < 0.001$), while diet was not associated with such decreases ($F_{2,34}$ = 3.065, $p = 0.06$) (Fig. 5C).

Two-way ANOVA revealed significant interaction effects of SDS and diet on the time spent in the interaction zone ($F_{2,34}$ = 18.911, $p < 0.001$) and corner zones ($F_{2,34}$ = 4.700, $p = 0.0165$) (Fig. 6A, B). The S-HFD-half group spent less time in the interaction zone and more time in the interaction zone when compared with the S-ND group, suggesting that the S-HFD-half group exhibited social avoidance. In the OF test, there were interaction effects of SDS and diet in the time spent in center areas ($F_{2,34}$ = 4.062, $p = 0.026$). Time spent in center areas was significantly lower in the S-HFD-half group than in the N-ND group, suggesting that the S-HFD-half diet induced anxiety-like behavior, relative to that observed in the S-ND group (Fig. 6C, D).

### 3.4 Inadequate supply of high-fat food does not alter SDS-induced plasma

**corticosterone levels**

Exposure to chronic stress activates the HPA axis, and a high-fat diet reduces HPA responses to repeated stressors [11,16]. Therefore, we measured plasma corticosterone levels, adrenal gland weight, and corticotropin-releasing hormone (CRH) expression in the hypothalamus (Fig. 7, 8) to analyze the effect of a high-fat diet and SDS on HPA axis activity. Two-way ANOVA revealed that SDS was associated with significant increases in plasma corticosterone levels in Experiment 1(SDS: $F_{1,30}$ = 6.471, p = 0.0164; diet: $F_{2,30}$ = 2.246, p = 0.1233) (Fig. 7A). However, in Experiment 2, we observed a significant interaction effect of SDS and diet ($F_{2,27}$ = 3.679, p = 0.038). No significant differences in corticosterone levels were observed between the S-HFD-half and N-HFD-half groups, although plasma corticosterone levels were higher in the S-ND group than in the N-ND group (Fig. 8A).

Exposure to SDS also significantly increased the level of CRH mRNA expression in the hypothalamus in Experiment 1 (SDS: $F_{1,30}$ =14.57, p < 0.001; diet: $F_{2,30}$ = 1.765, p = 0.1903) (Fig. 7B), whereas we observed a significant interaction effect of SDS and diet in Experiment 2 ($F_{2,32}$ = 3.964, p = 0.029) (Fig. 8B). Post hoc analysis revealed that CRH expression was significantly higher in the S-HFD-half group than in the N-ND group.

Chronic stress induced overactivation of the HPA axis as well as adrenal hypertrophy. Two-way ANOVA revealed significant interaction effects of stress and diet on adrenal gland volume in Experiments 1 ($F_{2,30}$ = 4.433, p = 0.0206) and 2 ($F_{2,34}$ = 7.003, p = 0.003) (Fig. 7C, 8C). Post hoc analysis revealed that adrenal gland volume was significantly higher in the S-ND group than in the N-ND group (Fig. 7C), whereas no significant difference was observed between the N-and S- HFD groups. In addition, no significant difference was observed between the S-HFD-half group and the N-ND group (Fig. 8C).

Taken together, our findings indicate that short-term intake of a high-fat diet does not alter HPA responses to repeated stressors, whereas lower intake of high-fat foods may alter responses to SDS without affecting the secretion of corticosterone.

### 3.5 Adequate intake of high-fat food during SDS alters cholesterol metabolism in the liver

Chronic stress disrupts the regulation of lipid synthesis in the liver [32]. To assess effects of stress and diet on lipid metabolism, we measured the expression levels of genes involved in lipid synthesis in the liver in Experiment 1 (Fig. 9).

Consumption of a high-fat diet increased CPT1a mRNA expression, which is

related to fatty acid beta-oxidation ($F_{2,30}$ = 8.097, p = 0.0016) (Fig. 9A). There was no significant difference in the expression of other lipid-related mRNA among the groups (Fig. 9B-E). In contrast, two-way ANOVA revealed a significant interaction effect of SDS and diet on the expression of Cyp7a1($F_{2,28}$ = 8.097, p = 0.0016). Post hoc analysis revealed that such expression was significantly higher in the S-HFD groups than in the S-ND group (Fig. 9F).

We next investigated whether consumption of a high-fat diet under SDS altered plasma cholesterol levels ($F_{2,28}$ = 5.724, p = 0.008) (Fig. 9G). Our results indicated that plasma cholesterol increased in the N-HFD-ad group, but not in the S-HFD-ad group.

Despite consumption of a high-fat diet, the S-HFD-half group exhibited no increases in CPT1a mRNA expression ($F_{2,32}$ = 5.386, p = 0.0096) (Fig. 10A). No significant differences in plasma cholesterol levels were observed among the groups ($F_{2,30}$ = 1.851, p = 0.1745) (Fig. 10B).

## 4. Discussion

The results of Experiment 1 indicated that mice of the S-HFD group did not exhibit social avoidance behavior. Although the intake of high-fat food was lower in the S-HFD-2h group than in the S-HFD-ad group, we observed similar effects of each diet on social avoidance behavior. However, no significant improvements in social avoidance behavior were observed in the S-HFD-half group in Experiment 2.

In the present study, we observed no significant differences in plasma corticosterone levels or CRH mRNA expression in the hypothalamus between the S-HFD-ad and the S-ND groups. This finding may indicate that short-term consumption of a high-diet (10 days in the present study) does not influence HPA axis responses to stress. Alternatively, preconditioning to daily palatable food may decrease HPA axis responses to acute stress.

Previous studies have suggested that palatable food intake decreased HPA axis activity, although these rodents received palatable food for 1 week [11,16] or 4 weeks [20] prior to experimental procedures such as restraint stress. However, we observed no decreases in HPA axis activity in the S-HFD group. Taken together, these results suggest that a history of palatable food consumption prior to stress exposure is important for decreasing HPA axis activity. To verify this assumption, further studies should evaluate the effect of a high-diet after exposure to stress, in order to determine whether alterations in feeding preference play a role in suppressing abnormal behavior induced by stress.

Palatable food has properties that promote dependence, which may lead to obesity and related psychological disorders such as a depression [25-27]. As expected, the HFD-ad groups exhibited significant increases in body weight and eWAT. However, the S-HFD-ad group exhibited decreased social avoidance when compared to the S-ND group. Some studies have reported that continued consumption of a high-fat diet induces anxiety-like behavior in rodents [26,27]. In these previous studies, the authors suggested that such behavior is associated with diet-induced obesity or type 2 diabetes. As we utilized a short-term SDS paradigm only, the diet utilized in the present study may have been insufficient for inducing obesity. Further studies should examine whether continuation of the paradigm would result in abnormal, obesity-related behaviors in the S-HFD-ad group.

As observed in the S-HFD-ad group, the S-HFD-2h group exhibited attenuated social avoidance behavior. However, in contrast to findings observed in the S-HFD-ad group, the S-HFD-2h group exhibited no increases in body weight or eWAT, suggesting that a high-fat diet can suppress social avoidance behavior without increases in body

weight.

Some studies have reported that sporadic, limited access to palatable food results in binge-type eating, which may increase under conditions of stress [35,36]. In the present study, we observed no significant differences in total calorie intake between the N-HFD-2h and S-HFD-2h groups. Moreover, HPA axis activity was normal in the S-HFD-2h group. These results suggest that binge-type eating did not occur in either group, and that consumption of a high-fat diet does not induce negative effects on HPA axis activity related to binge-type eating.

The results of Experiment 1 indicated that mice subjected to a high-fat diet following SDS exhibited reduced social avoidance behavior, without alterations in HPA axis responses. Such results led us to hypothesize that a more restrictive high-fat diet would also suppress SDS-induced social avoidance. Contrary to our hypothesis, social avoidance behavior was similar between the S-HFD-half and S-ND groups. Interestingly, the S-HFD-half group also exhibited anxiety-like behavior in the OF test, which was not observed in the S-ND group. Moreover, the S-HFD-half group exhibited no increases in plasma corticosterone levels or adrenal grand weight, while this group exhibited increases in CRH mRNA in the hypothalamus. These results suggest that, while the S-HFD-half group exhibited the potential for alterations in responses to SDS, they did not experience alterations in the production and/or secretion of corticosterone. Generally, corticosterone deficiency under conditions of stress induces abnormal behavior. Indeed, rodents subjected to bilateral adrenalectomy (ADX) to negate the effect of corticosterone exhibit increases in anxiety-like behavior [37]. In such rats, levels of CRH mRNA are increased in the hypothalamus, without concomitant increases in plasma corticosterone levels [35,38]. With the exception of body weight loss, which is common in ADX rodents, results were similar in S-HFD-half mice of the present study [38,39]. Future studies should aim to determine the mechanisms by which SDS-induced increases in corticosterone are suppressed in S-HFD-half mice.

In this study, adrenal gland weight increased in Experiment 2, but not in Experiment 1 in the S-HFD-2h group. This discrepancy is thought to be influenced by the difference in the aggressive character of aggressor ICR mice and/or the vulnerability of individual experimental C57BL/6 mice. However, S-HFD-2h mice showed normal social behavior, suggesting that adrenal gland weight might be not important for alteration in social behavior although chronic HPA axis activation enlarges adrenal gland. In addition, hypothalamic CRH neurons have diversity [40], suggesting that the responded CRH cells by stress might be not necessary for HPA axis [41]. Contradicted observation in which higher CRH mRNA in HFD-half group despite no increase in

plasma corticosterone and adrenal gland weight might reflect the CRH neuron's dissociated function. CRH has also central contrasting effects on food intake; one is counteracted function against feeding-stimulated neuropeptides [42], the other is activation for selected carbohydrate intake after fasting [43]. These pleiotropic effects of CRH may make us difficulty to fully understand the significance in CRH expression, adrenal gland weigh, plasma corticosterone and HPA axis activity under stress condition. Further investigations are needed.

On the other hand, CRH-expressing neuron in the paraventricular hypothalamus also expresses arginine vasopressin (AVP), and both CRH and AVP secretion are stimulated by various stress. AVP potentiates the stimulatory effect of CRH [44]. In our study, the CRH mRNA level was significantly increased by stress, but not by HFD. Similarly, the AVP mRNA was increased by SDS ($F_{1,30}$ = 7.98, p = 0.008, data not shown) while HFD intake was not associated with such an increase ($F_{2,30}$ = 1.159, p = 0.327). These data suggest that SDS induced a normal stress response, and in our experiment, the high-fat diet might not have interfered with the stress response.

We also evaluated the effects of a high-fat diet and stress on mRNA expression associated with lipid metabolism. In Experiment 1, both S-HFD groups exhibited increased levels of Cyp7a1 mRNA in the liver (related to cholesterol metabolism) when compared with the S-ND group. Cyp7a1 is required for the conversion of cholesterol to bile acid, increases in Cyp7a1 mRNA expression occur in response to increases in cholesterol levels. Thus, our data suggest that consumption of a high-fat diet after exposure to SDS influences cholesterol metabolism. Previous studies have reported that rodents subjected to a high-fat diet exhibit increase in plasma cholesterol levels [45]. In our study, the S-HFD groups exhibited no such increases in plasma cholesterol. However, increases in plasma cholesterol were observed in the N-HFD-ad group, which was consistently subjected to a high-fat diet.

Steroid hormones are typically eliminated by inactivating metabolic transformations and via excretion in urine or bile [46]. Bile acid is involved in regulating glucose and lipid metabolism in the liver, as well as energy expenditure [47-49]. These previous reports have indicated that there may be complex interactions between glucocorticoids and bile acid homeostasis. One recent study reported that chronic stress impairs the intestinal absorption of bile acids, although apparent bile acid depletion did not increase in CYP7A1-mediated bile acid synthesis [50]. When taken with these findings, our results support the notion that consumption of a high-fat diet under stress induces cholesterol metabolism and/or progresses the metabolism of corticosterone. Further research is required to reveal the mechanisms underlying

cholesterol metabolism during stress, particularly with regard to bile acid synthesis and excretion.

Despite consumption of a high-fat diet, the S-HFD-half group exhibited no increases in the mRNA expression of Cyp7a1 or plasma corticosterone levels. These results highlight the importance of corticosterone secretion in the stress response, and suggest the necessity of elevated corticosterone levels and increased cholesterol metabolism in the liver.

SDS is one of the useful animal models for the experiment of depression [29]. Our SDS experiment did not induce a depressive-like behavior on TS test, although we observed the social avoidance behavior in same mice. The SDS experiment was consistent of a short-time physical session after which the subject mouse's home cage was separated from the aggressor mice, suggesting that our SDS experiment might have produced mild stress. Moreover, since we did not evaluate the effect of high-fat diet on depressive-like behavior under stress conditions other than SDS, it is unclear whether a high-fat diet is effective in improving depression. Further experiments are needed to understand the effect of high-fat diet on depression using various stress conditions such as restraint stress.

## 5. Conclusions

In the present study, we investigated whether the amount of high-fat intake influenced behavior during periods psychosocial stress. Our results demonstrate that eating a high-fat diet may attenuate stress, but that this benefit disappears with insufficient intake of high-fat foods. The benefits of a high-fat diet under SDS may be related to cholesterol metabolism in the liver.

## Acknowledgments

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## Figure legends

**Fig 1. Schematic representation of the SDS paradigm.** (A): Feeding schedule in Experiment 1. SDS: social defeat stress; HFD: high-fat diet; ND: normal diet. (B): Experimental mice were separated into six groups in Experiment 1: N-ND, S-ND, N-HFD-ad, S-HFD-ad, N-HFD-2h, and S-HFD-2h. All S-group mice were placed into an aggressor's home cage and experienced physical contact with the aggressive ICR mouse for 2.5 min. In contrast, N-group mice were placed into their own cages. All HFD groups were provided with high-fat chow in their home cages after each SDS exposure (bottom panel), whereas S-ND groups were placed into their home cages without high-fat chow (upper panel).

**Fig 2. Schematic representation of the SDS paradigm in Experiment 2**. SDS: social defeat stress; HFD: high-fat diet.

**Fig 3. Effect of HFD and/or SDS on food intake and body weight in Experiment 1.** Total calories consumed in each experimental group during SDS (A). Changes in body weight at Day 6 (for 5 days) and Day 11 (for 10 days) relative to that at zeitgeber time 0 (ZT 0) (B). Weight of the epididymal white adipose tissue corrected by body weight (C). Data are presented as the mean ± standard error (SE), n=6. Two-way ANOVA, $p < 0.01$: ** (Bonferroni test), $p < 0.01$: $^{\#\#}$ vs N-ND, $p < 0.01$: $^{++}$ vs S-ND. SDS: social defeat stress; HFD: high-fat diet.

**Fig 4. Effect of a HFD and/or SDS on behavioral results in Experiment 1.** Time spent in the interaction zone (A) and corner zone (B) during the second social interaction test. Total distance traveled (C) and time spent in the central zone (D) in the open-field test. Ratios of the distance (E) and time spent in the two chambers (F) in the light–dark test. Total immobility time (G) in the tail suspension test. Data are presented as the mean ± standard error (SE), n=6–9. Two-way ANOVA, $p < 0.05$: *, $p < 0.01$: ** (Bonferroni test). SDS: social defeat stress; HFD: high-fat diet.

**Fig 5. Effect of HFD and/or SDS on food intake and body weight in Experiment 2**. Total calories consumed in each experimental group during SDS (A). Changes in body weight at Day 6 (for 5 days) and Day 11 (for 10 days) relative to that at zeitgeber time 0 (ZT 0) (B). Weight of the epididymal white adipose tissue corrected by body weight (C). Data are presented as the mean ± standard error (SE), n=6-8. Two-way ANOVA,

$p < 0.05$: #, $p < 0.01$: ## vs N-ND. SDS: social defeat stress; HFD: high-fat diet.

**Fig 6. Effect of a half-volume HFD on behavior in Experiment 2.** Time spent in the interaction zone (A) and corner zone (B) during the second social interaction test. Total distance traveled (C) and time spent in the central zone (D) in the open-field test. Data are presented as the mean ± standard error (SE), n=6–8. Two-way ANOVA, $p < 0.05$: *, $p < 0.01$: ** (Bonferroni test). HFD: high-fat diet.

**Fig 7. Effect of HFD and/or SDS on activity of the hypothalamic-pituitary-adrenal (HPA) axis in Experiment 1.** The concentration of plasma corticosterone (A). Corticotropin-releasing hormone (CRH) mRNA levels in the hypothalamus corrected by β-actin levels (B). Weight of the adrenal gland corrected by body weight (C). Each sample was collected at Day 15, and plasma concentrations of corticosterone were assessed via ELISA. Data are presented as the mean ± standard error (SE), n=6. Two-way ANOVA, $p < 0.05$: *, $p < 0.01$: ** (Bonferroni test). SDS: social defeat stress; HFD: high-fat diet.

**Fig 8. Effect of a half-volume HFD on activity of the hypothalamic-pituitary-adrenal (HPA) axis in Experiment 2.** The concentration of plasma corticosterone (A). Corticotropin-releasing hormone (CRH) mRNA levels in the hypothalamus corrected by β-actin levels (B). Weight of the adrenal gland corrected by body weight (C). Data are presented as the mean ± standard error (SE), n=6–8. Two-way ANOVA, $p < 0.05$: *, $p < 0.01$: ** (Bonferroni test). HFD: high-fat diet.

**Fig 9. Effect of HFD and/or SDS on mRNA expression in the liver and plasma cholesterol levels in Experiment 1.** Lipid metabolism-related mRNA expression in the liver (A-F). Concentration of plasma cholesterol (G). Data are presented as the mean ± SE, n=6. Two-way ANOVA, $p < 0.05$: *, $p < 0.01$: ** (Bonferroni test), $p < 0.01$: ## vs N-ND, $p < 0.05$: +, $p < 0.01$: ++ vs S-ND. SDS: social defeat stress; HFD: high-fat diet.

**Fig 10. Effect of a half-volume HFD on mRNA expression in the liver and plasma cholesterol levels in Experiment 2.** Cholesterol metabolism-related mRNA expression in the liver (A). Concentration of plasma cholesterol (B). Data are presented as the mean ± SE, n=6-8. Two-way ANOVA, $p < 0.05$: *, $p < 0.01$: ** (Bonferroni test). HFD: high-fat diet.

## Table legends

### Table 1. List of primers.

| Gene | Forward (5′–3′) | Reverse (3′–5′) | Accession number |
|---|---|---|---|
| Cpt1a | CCAGGCTACAGTGGGACATT | GAACTTGCCCATGTCCTTGT | NM_013495 |
| Ppara | CACGCATGTGAAGGCTGTAA | CAGCTCCGATCACACTTGTC | NM_001113418 |
| Ppargc1a | CAGTCGCAACATGCTCAAG | TGGGGTCATTTGGTGACTCT | NM_008904 |
| Srebf1 | ACAAGATTGTGGAGCTCAAAGAC | GCGCAAGAGCAGATTTATT | NM_001313979 |
| Fasn | GCTGCTGTTGGAAGTCAGC | AGTGTTCGTTCCTCGGAGTG | NM_007988 |
| Cyp7a1 | GGGATTGCTGTGGTAGTGAGC | GGTATGGAATCAACCCGTTGTC | NM_007824 |
| Crh | GGAGGCATCCTGAGAGAAGTC | CATGTTAGGGGCGCTCTC | NM_205769 |
| actb | CTAAGGCCAACCGTGAAAAG | ACCAGAGGCATACAGGGACA | NM_007393 |

### Table 2. Amount of food intake during SDS for Experiment 1 (mean ± SEM, n = 6).

| | ND | | HFD-ad | | HFD-2h | |
|---|---|---|---|---|---|---|
| **Average of intake** | N- | S- | N- | S- | N- | S- |
| Total intake (kcal) | 11.2 ± 0.3 $^{a}$ | 10.4 ± 0.5 $^{a}$ | 14.6 ± 0.4 $^{b}$ | 12.7 ± 0.4 $^{c}$ | 10.0 ± 0.2 $^{a}$ | 9.9 ± 0.2 $^{a}$ |
| 1-5 day (kcal) | 11.7 ± 0.2 $^{ac}$ | 9.8 ± 0.7 $^{a}$ | 16.3 ± 0.4 $^{b}$ | 13.5 ± 0.6 $^{c}$ | 9.9 ± 0.3 $^{a}$ | 9.8 ± 0.3 $^{a}$ |
| 6-10 day (kcal) | 10.7 ± 0.4 $^{a}$ | 11.0 ± 0.4 $^{xy}$ | 12.9 ± 0.5 $^{b}$ | 11.9 ± 0.4 $^{x}$ | 10.2 ± 0.2 $^{a}$ | 10.1 ± 0.1 $^{y}$ |
| High fat diet intake (g) | - | - | 2.8 ± 0.1 $^{a}$ | 2.4 ± 0.1 $^{b}$ | 1.7 ± 0.1 $^{c}$ | 1.3 ± 0.1 $^{d}$ |

Significant differences are indicated using different superscript letters ($p < 0.05$). For food intake at Days 6-10, we observed no differences in the stress × diet interaction. Thus, only the main effect of diet is indicated using superscript letters (N-: a, b, c; S-:x, y, z). SDS: social defeat stress.

### Table 3. Amount of food intake during SDS for Experiment 2 (mean ± SEM, n = 6-8).

| | ND | | HFD-2h | | HFD-half | |
|---|---|---|---|---|---|---|
| **Average of intake** | N- | S- | N- | S- | N- | S- |
| Total intake (kcal) | 10.8 ± 0.2 $^{a}$ | 12.7 ± 0.2 $^{b}$ | 11.8 ± 0.4 $^{ab}$ | 11.2 ± 0.5 $^{ab}$ | 11.6 ± 0.3 $^{ab}$ | 10.4 ± 0.5 $^{a}$ |
| 1-5 day (kcal) | 11.0 ± 0.5 $^{ab}$ | 12.6 ± 0.2 $^{b}$ | 11.9 ± 0.4 $^{ab}$ | 11.0 ± 0.4 $^{ab}$ | 11.3 ± 0.4 $^{ab}$ | 10.3 ± 0.7 $^{a}$ |
| 6-10 day (kcal) | 10.6 ± 0.3 $^{a}$ | 12.7 ± 0.3 $^{b}$ | 11.8 ± 0.4 $^{ab}$ | 11.5 ± 0.5 $^{ab}$ | 11.9 ± 0.3 $^{ab}$ | 10.6 ± 0.3 $^{a}$ |

Significant differences are indicated using different superscript letters ($p < 0.05$).

**Figure**

# Figure1

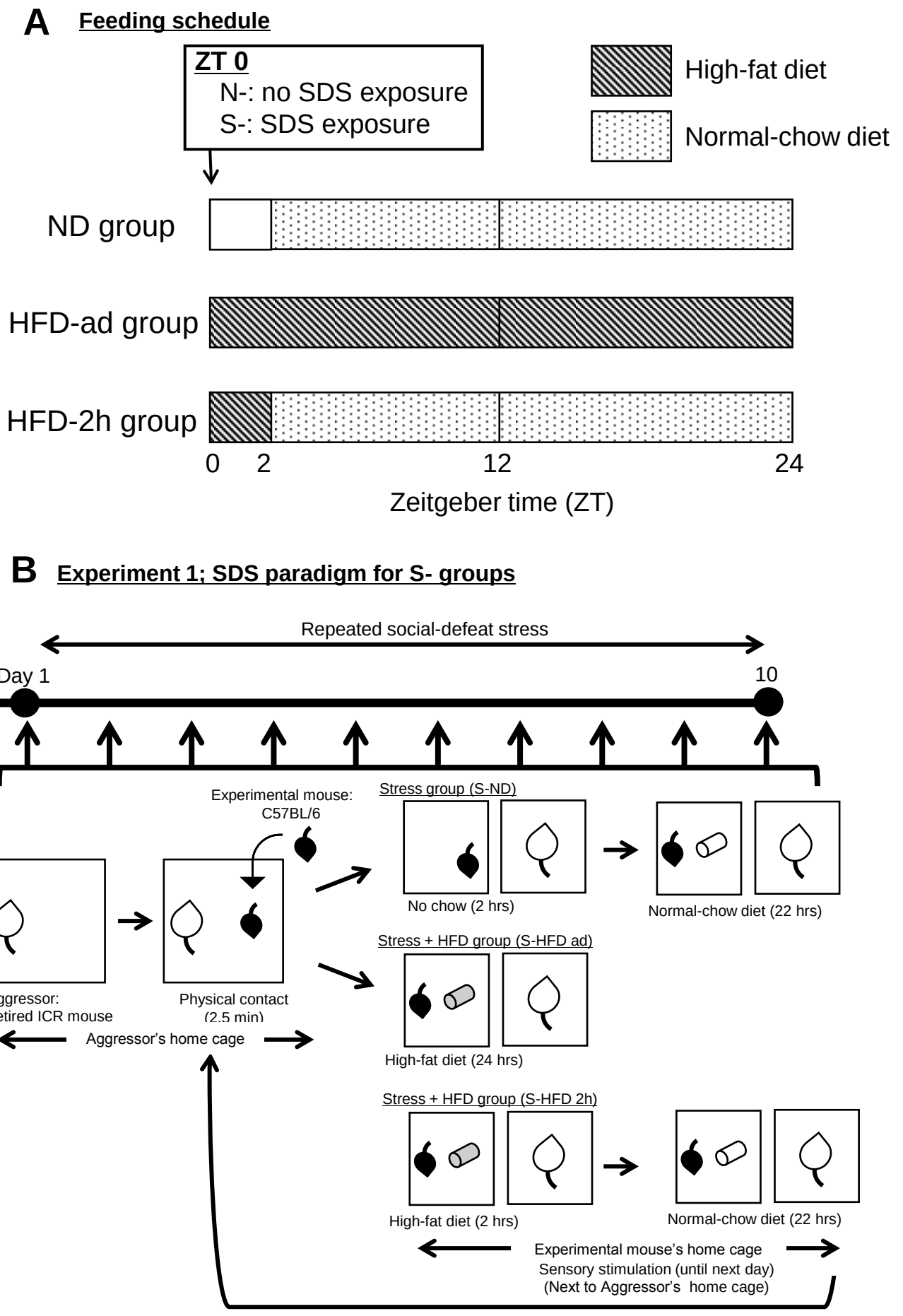


Figure2

**Experiment 2; SDS paradigm for S-HFD-half**

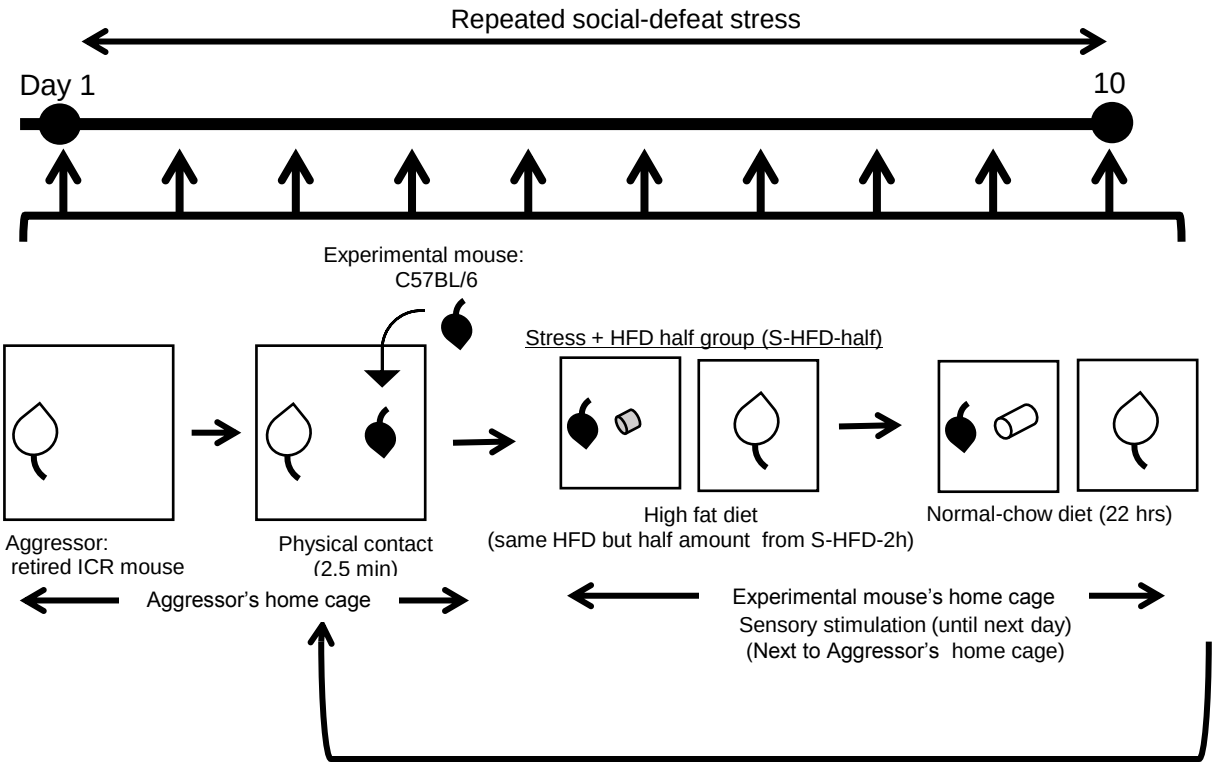
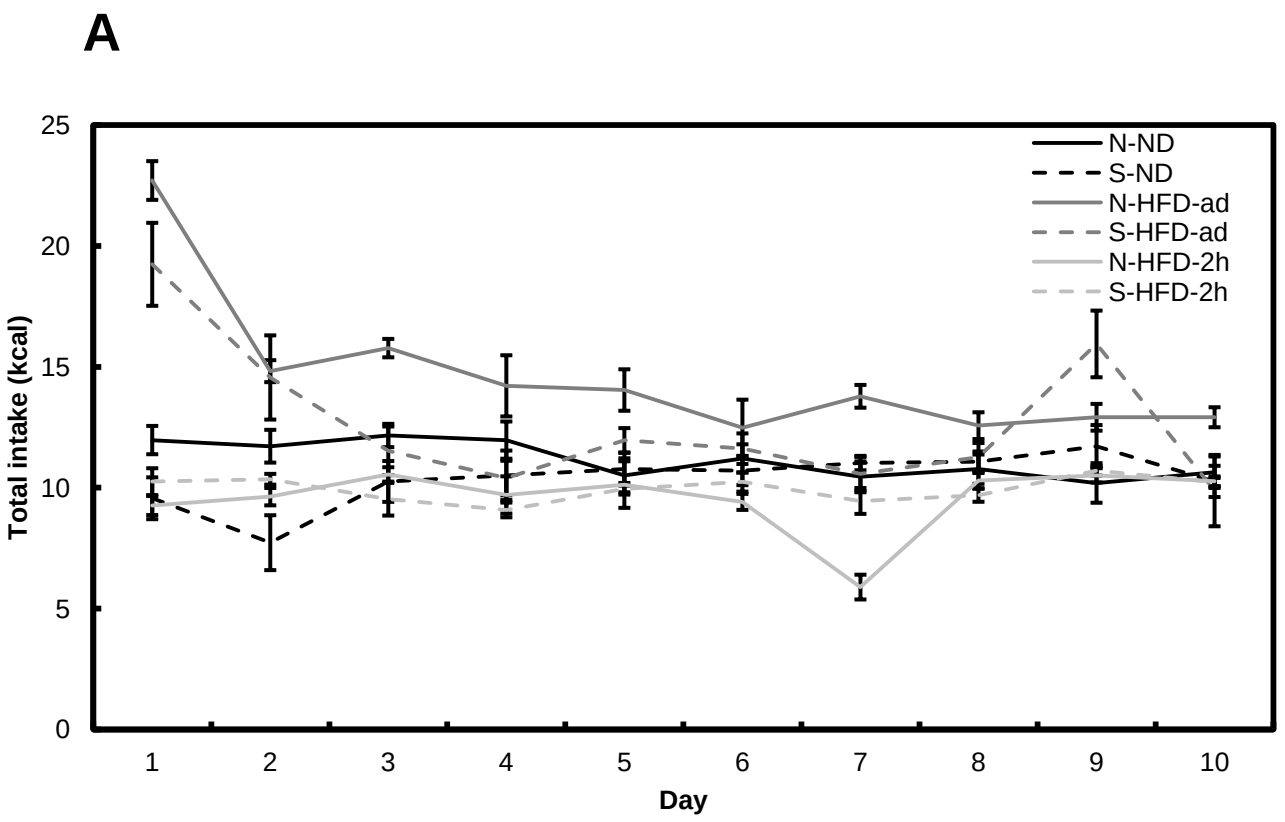
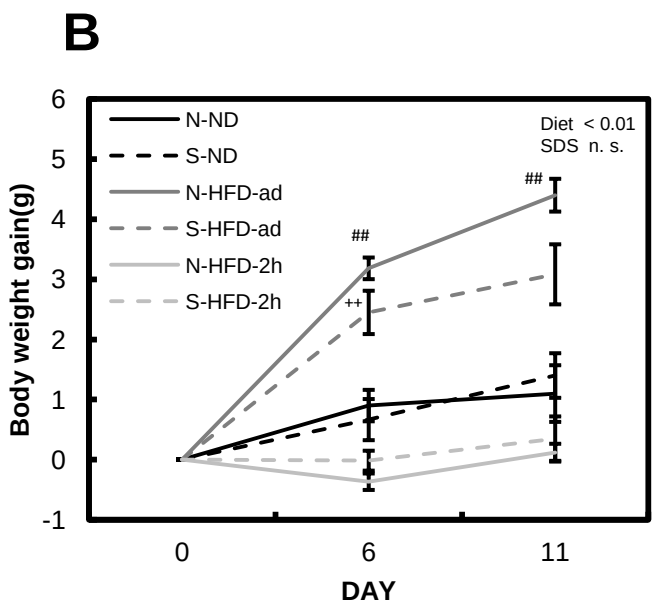


Figure 3


A
Total intake (kcal)
Day
N-ND
S-ND
N-HFD-ad
S-HFD-ad
N-HFD-2h
S-HFD-2h


B
Body weight gain(g)
DAY
Diet < 0.01
SDS n. s.
##
++

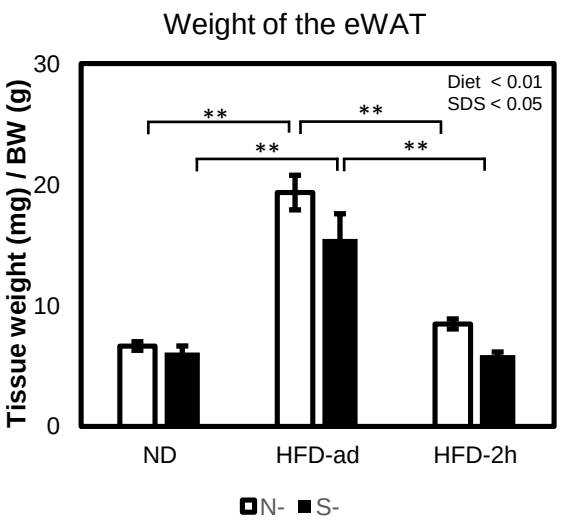

C
Weight of the eWAT
Tissue weight (mg) / BW (g)
Diet < 0.01
SDS < 0.05
**
ND
HFD-ad
HFD-2h
N-
S-

Figure 4

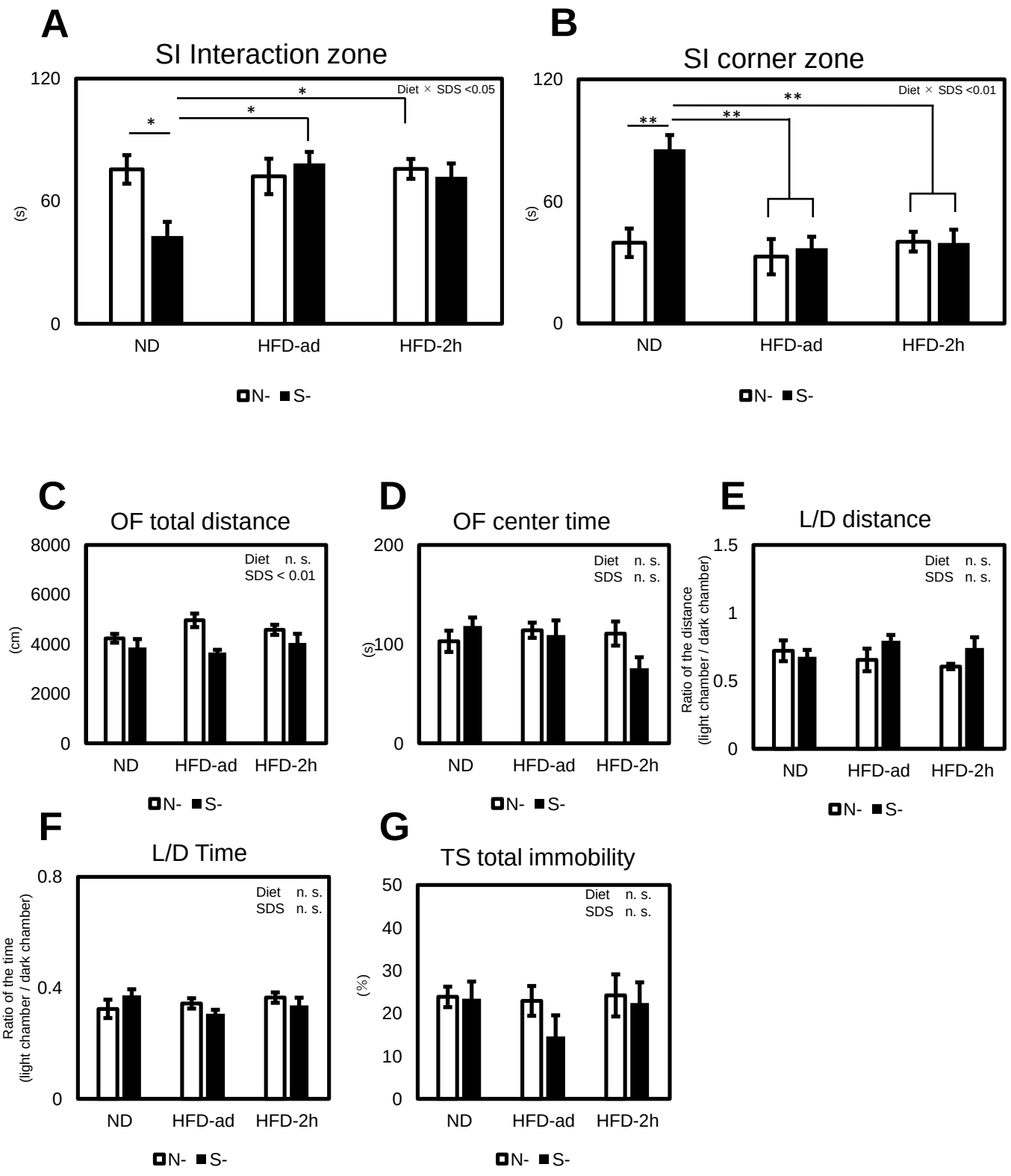
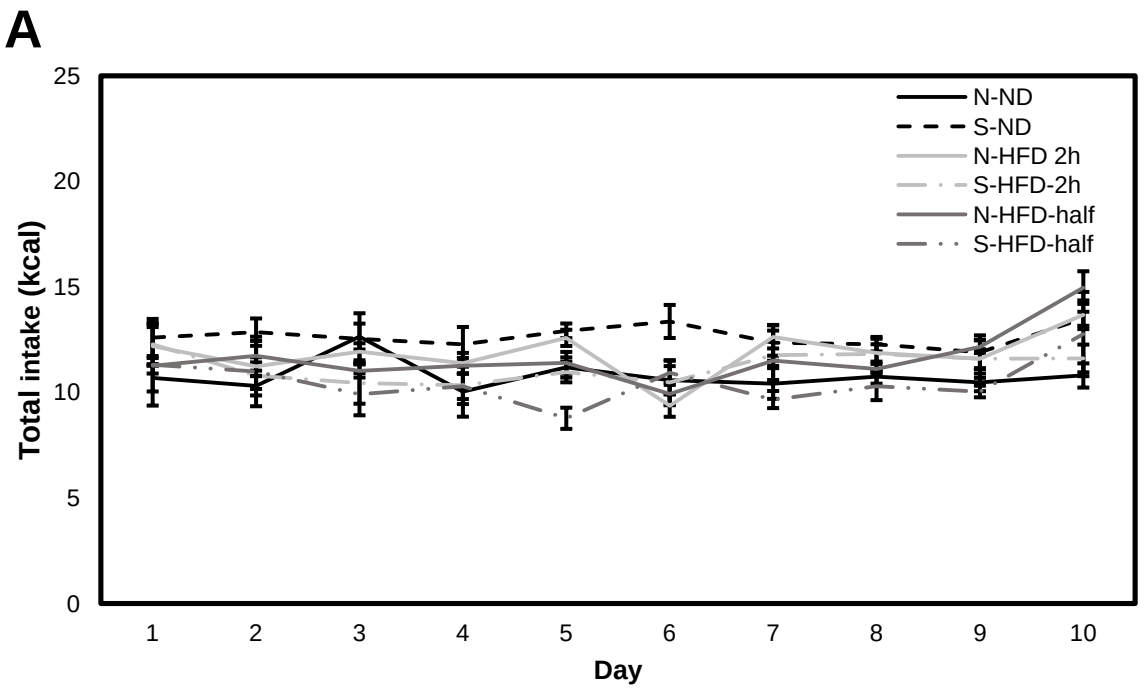
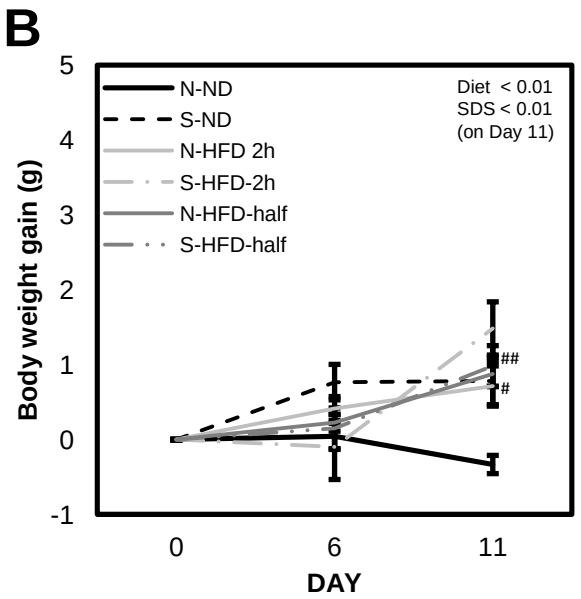

A
SI Interaction zone
Diet × SDS <0.05
120
60
0
(s)
ND
HFD-ad
HFD-2h
N- S-
B
SI corner zone
Diet × SDS <0.01
C
OF total distance
Diet n. s.
SDS < 0.01
8000
6000
4000
2000
(cm)
D
OF center time
Diet n. s.
SDS n. s.
200
100
E
L/D distance
1.5
1
0.5
Ratio of the distance
(light chamber / dark chamber)
F
L/D Time
0.8
0.4
Ratio of the time
(light chamber / dark chamber)
G
TS total immobility
50
40
30
20
10
(%)

Figure 5


A
Total intake (kcal)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
Day
N-ND
S-ND
N-HFD 2h
S-HFD-2h
N-HFD-half
S-HFD-half


B
Body weight gain (g)
5
4
3
2
1
0
-1
0
6
11
DAY
N-ND
S-ND
N-HFD 2h
S-HFD-2h
N-HFD-half
S-HFD-half
Diet < 0.01
SDS < 0.01
(on Day 11)
##
#

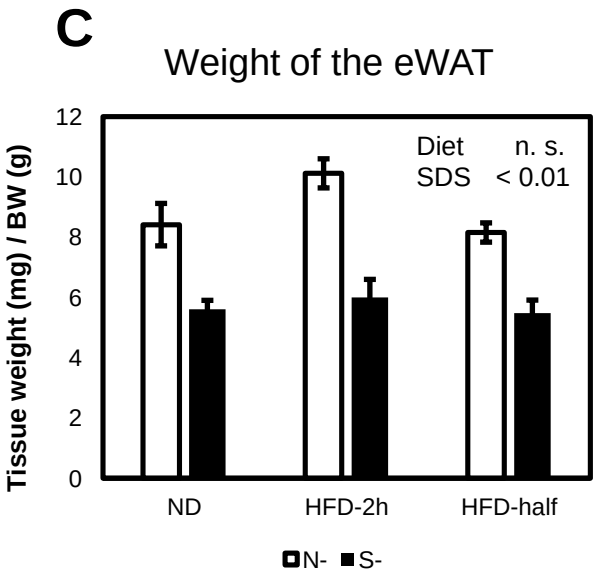

C
Weight of the eWAT
Tissue weight (mg) / BW (g)
12
10
8
6
4
2
0
Diet n. s.
SDS < 0.01
ND
HFD-2h
HFD-half
N-
S-

Figure 6

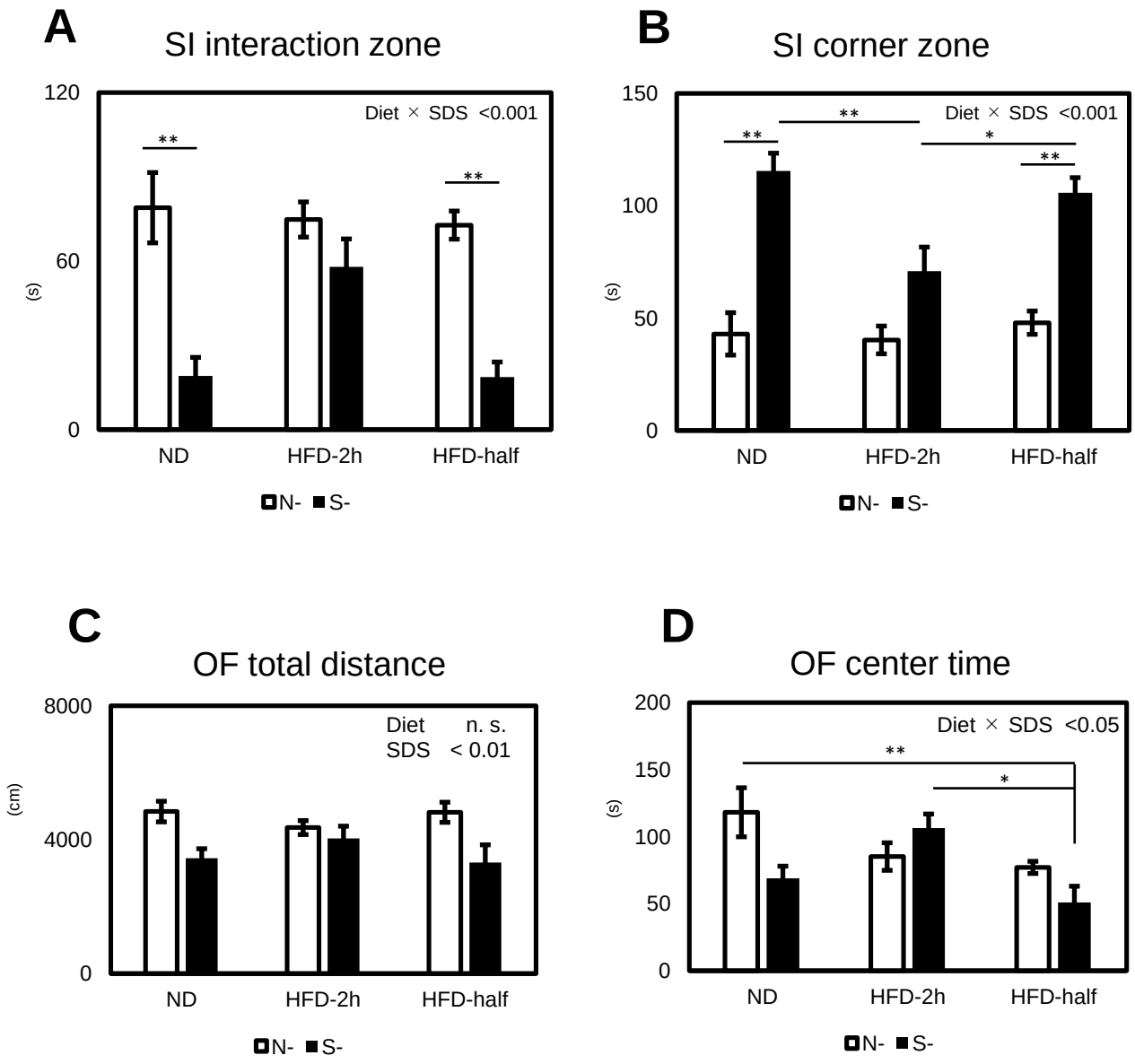

A
SI interaction zone
120
60
0
(s)
Diet × SDS <0.001
**
**
ND
HFD-2h
HFD-half
N- S-
B
SI corner zone
150
100
50
0
(s)
Diet × SDS <0.001
**
**
*
**
ND
HFD-2h
HFD-half
N- S-
C
OF total distance
8000
4000
0
(cm)
Diet n. s.
SDS < 0.01
ND
HFD-2h
HFD-half
N- S-
D
OF center time
200
150
100
50
0
(s)
Diet × SDS <0.05
**
*
ND
HFD-2h
HFD-half
N- S-

Figure 7

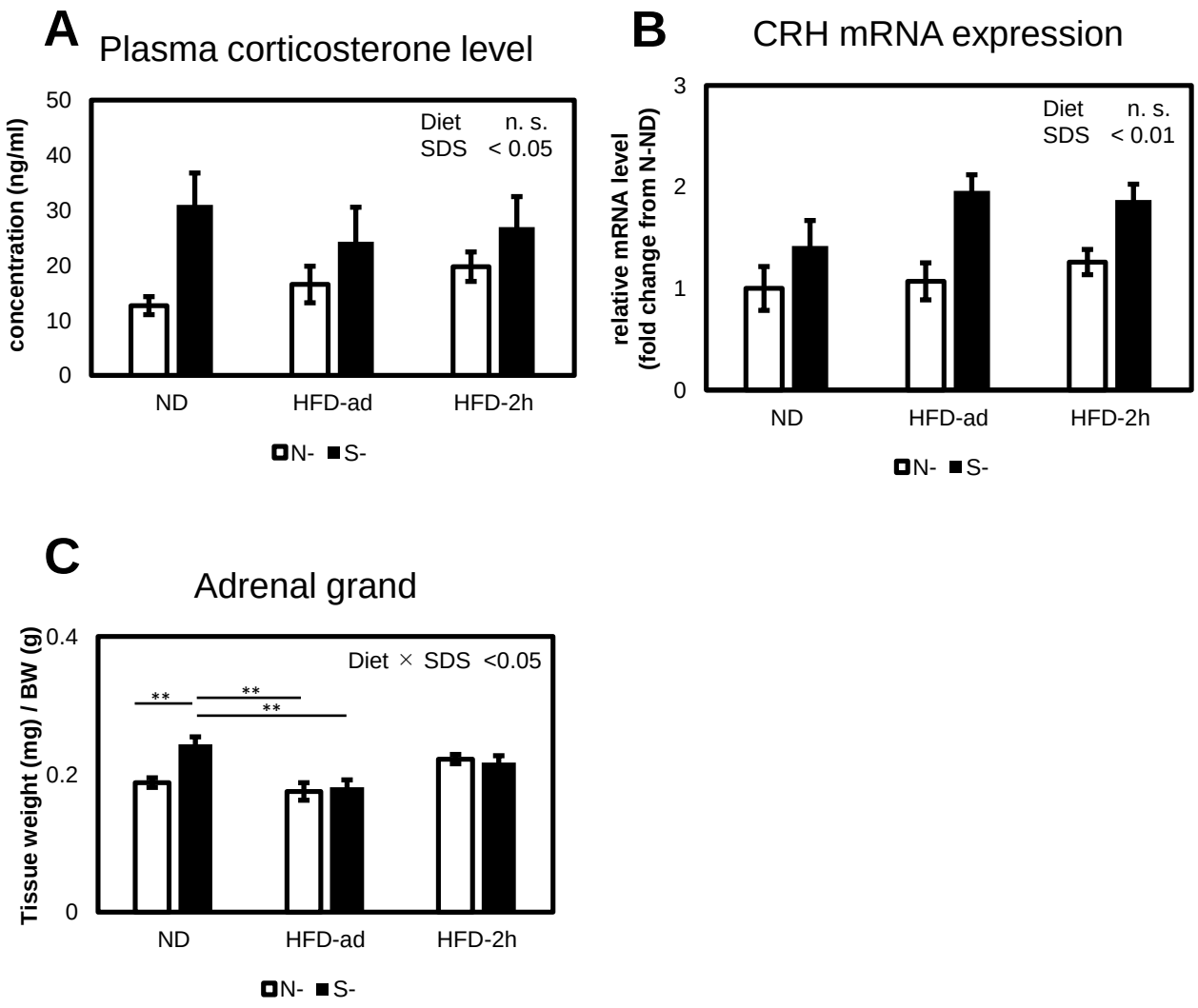

A
Plasma corticosterone level
concentration (ng/ml)
50
40
30
20
10
0
Diet n. s.
SDS < 0.05
ND
HFD-ad
HFD-2h
N- S-
B
CRH mRNA expression
relative mRNA level
(fold change from N-ND)
3
2
1
0
Diet n. s.
SDS < 0.01
ND
HFD-ad
HFD-2h
N- S-
C
Adrenal grand
Tissue weight (mg) / BW (g)
0.4
0.2
0
Diet × SDS <0.05
**
**
**
ND
HFD-ad
HFD-2h
N- S-

Figure 8

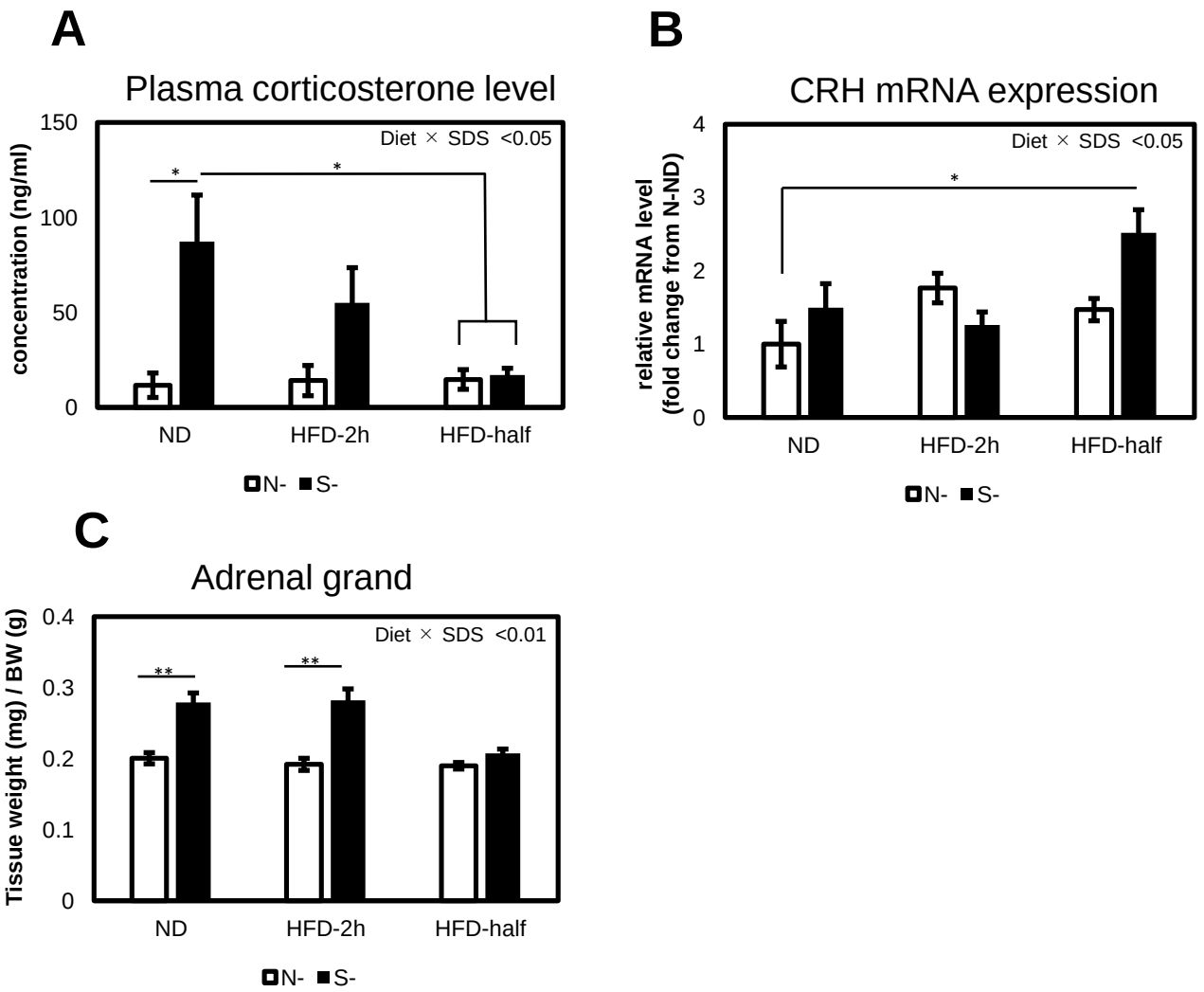

A
Plasma corticosterone level
concentration (ng/ml)
150
100
50
0
Diet × SDS <0.05
*
*
ND
HFD-2h
HFD-half
N- S-
B
CRH mRNA expression
relative mRNA level (fold change from N-ND)
4
3
2
1
0
Diet × SDS <0.05
*
ND
HFD-2h
HFD-half
N- S-
C
Adrenal grand
Tissue weight (mg) / BW (g)
0.4
0.3
0.2
0.1
0
Diet × SDS <0.01
**
**
ND
HFD-2h
HFD-half
N- S-

Figure 9

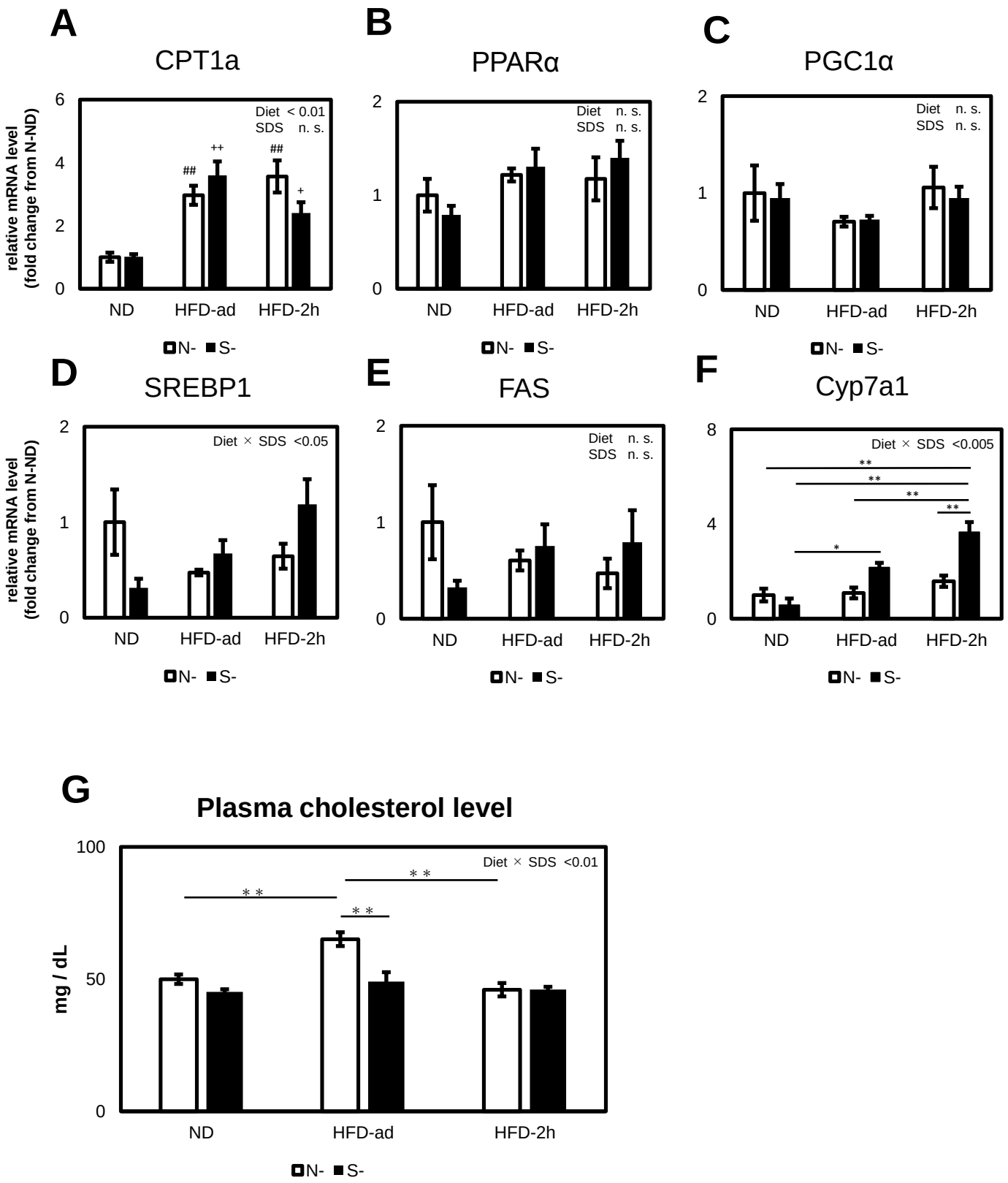
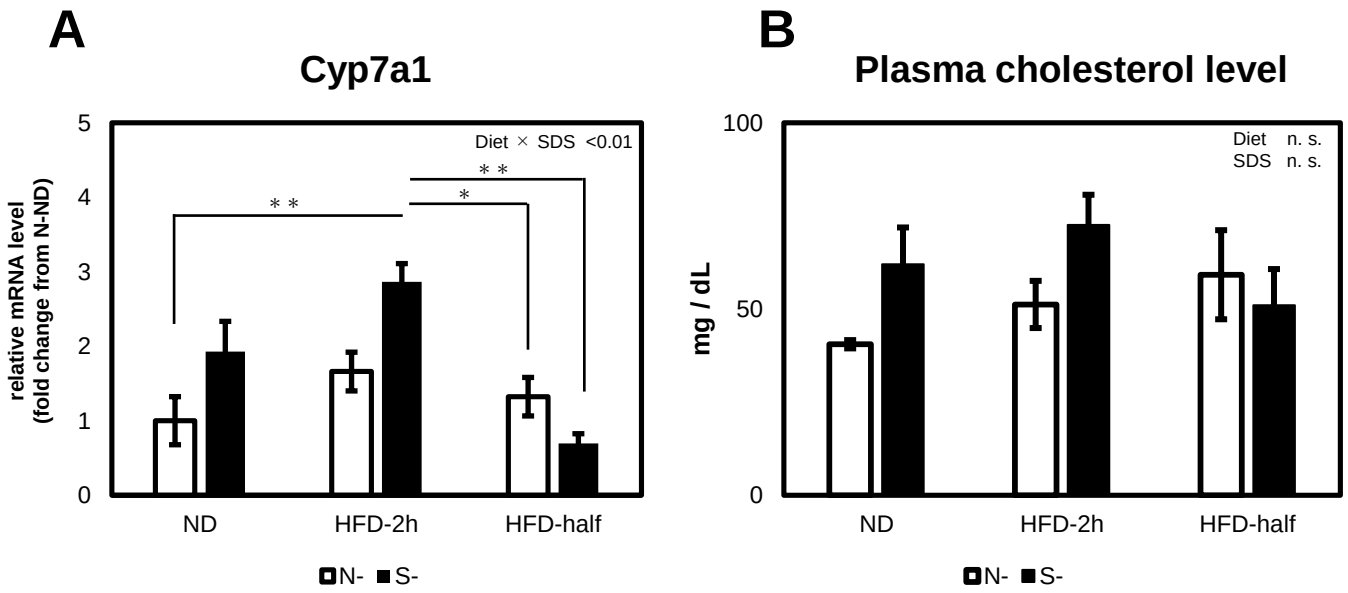

A
CPT1a
relative mRNA level (fold change from N-ND)
Diet < 0.01
SDS n. s.
##
++
##
+
ND
HFD-ad
HFD-2h
N- S-
B
PPARα
Diet n. s.
SDS n. s.
C
PGC1α
Diet n. s.
SDS n. s.
D
SREBP1
Diet × SDS <0.05
E
FAS
Diet n. s.
SDS n. s.
F
Cyp7a1
Diet × SDS <0.005
G
Plasma cholesterol level
mg / dL
Diet × SDS <0.01

Figure 10


A
Cyp7a1
relative mRNA level
(fold change from N-ND)
5
4
3
2
1
0
Diet × SDS <0.01
* *
* *
*
ND
HFD-2h
HFD-half
N- S-
B
Plasma cholesterol level
mg / dL
100
50
0
Diet n. s.
SDS n. s.
ND
HFD-2h
HFD-half
N- S-