

Continuous and Discontinuous Adherence to the Sticky Japanese Diet on Adiponectin and Leptin Regulation

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Abstract

Background: In order to evaluate the regulational role of adiponectin and leptin on obesity, we examined the significance of daily adherence to the Sticky Japanese Diet (SJD) on the preventions of obesity and metabolic syndrome by assessing the fat adiponectin/leptin and plasma leptin levels using normal and high-fat diet (HFD) mice.

Methods: Five-week-old male ICR strain mice were placed in individual cages and randomly divided into eight groups based on feeding of SJD or not. At the end of each treatment period, plasma triglyceride, cholesterol, and leptin levels were measured by biochemical analysis. The adiponectin and leptin levels in adipose tissue were measured using RT-PCR.

Results: In normal mice, an eight-week treatment of SJD increased adiponectin levels in adipose tissue. However, four weeks after discontinuing the SJD, these levels decreased. Furthermore, continuous feeding of the SJD for eight weeks increased plasma leptin levels but not that of adipose tissue. And then, discontinuing the SJD for more than eight days resulted in a decrease in plasma leptin levels. In HFD mice, an increase in body weight and visceral fat was seen at eight days after discontinuing the SJD which was undertaken for a total of eight weeks. At this time, the plasma leptin levels in these mice were substantially higher than in normal mice, although these levels were getting to be decreased over four weeks.

Conclusion: This study revealed that discontinuous adherence to the SJD induced decreases in both adiponectin and leptin levels. Together with our previous publication, this paper suggests that daily adherence to the SJD might be essential for maintaining good health.

Keywords: NuruNeba (Sticky Japanese Diet); Washoku (Japanese foods); normal diet; high-fat diet; leptin; adiponectin; obesity; mice

Abbreviations: SJD: Sticky Japanese Diet; HFD: High-Fat Diet; CE-2: CLEA Rodent Diet CE-2; TG: Triglyceride; UCP1: Uncoupling Protein-1; BAT: Brown Adipose Tissue

Introduction

It has been reported that the daily consumption of commercially available Japanese food products (Sticky Japanese Diet; SJD) containing ten kinds of sticky and slimy food components, which include a root kelp (*Laminariaceae*), wakame (*Undaria pinnatifida*), agar (generic name of raw material-*Gelidiaceae*), white cloud ear (*Tremella fuciformis*), shiitake (*Lentinula edodes*), nameko (*Pholiota nameko*), okra (*Abelmoschus esculentus*), mekabu (root of *Undaria pinnatifida*), cut tororo (*Dioscorea japonica*), and shimeji (*Hypsizygus tessellatus*). Significantly reduced the gain of body weight and visceral fat mass in high-fat diet (HFD; High Fat Diet 32: Japan CLEA, Tokyo) mice. The anti-obesity effects of SJD relate to the regulation of leptin levels in the adipose tissue of HFD mice [1]. These results indicated that adherence to SJD might play a preventative role against metabolic syndrome. Moreover, we reported that SJD

in normal diet (CE-2; Japan CLEA, Tokyo) mice significantly increased adiponectin levels; however, leptin levels in the adipose tissue did not increase. Two independent reports by Tawara et al. and Hirokawa et al. suggest that increased adiponectin levels, under normal conditions, may be more effective in preventing obesity rather than impeding leptin-resistance in adipose tissue [1, 2].

In order to clarify the mechanism of SJD activity on obesity and other related diseases, we investigated the anti-obesity effects of continuous SJD consumption on normal mice, which were fed either the CE-2 or HFD. Furthermore, normal mice were continuously fed SJD until an increase in the adiponectin levels of the adipose tissue was detected, and the changes in body weight were measured. Additionally, normal mice continuously consumed SJD for two months and, subsequently, the presence or absence of an increase in adiponectin was elaborated. Once SJD

5 min at 85 °C. Amplification of cDNA was performed using primers and Light Cycler® 480(Roche Life Science, Basel, Switzerland) according to the TaqMan™ Probe Method (Applied Biosystems, Foster City, CA, USA)(Table 1) at the following conditions: 10 min at 95 °C, 40 to 50 cycles for 20–30 s at 60 °C, 20 to 30 cycles.

Data Analysis

All results were expressed as mean with corresponding standard error. The Tukey-Kramer test and t-test were used for the analysis of significant differences between the groups. A value of $p < 0.05$ or less was considered statistically significant. These data were analyzed using Excel software statistics.

Table1: Primer used for each marker in Real Time PCR

Gene	Universal Probe Library	Forward (Left)	Reverse (Right)
GAPDH	#9	AGCTTGTCATCAACGGGAAG	TTTGATGTGGGGTCTCG
ADIPONECTIN	#95	ACCGGCAGACAAGAGCAG	TGGTGGGTACAACACCACTC
LEPTIN	#45	GTGGTGGCTGGTGTCAGATT	TTGATGAGGTGACCAAGGTG

Results

Effects of SJD on the Changes in Body Weight

Figure 2A shows the changes in the bodyweight of the mice in groups ⑤C→C and ⑥C→H. The body weight changes of the mice in

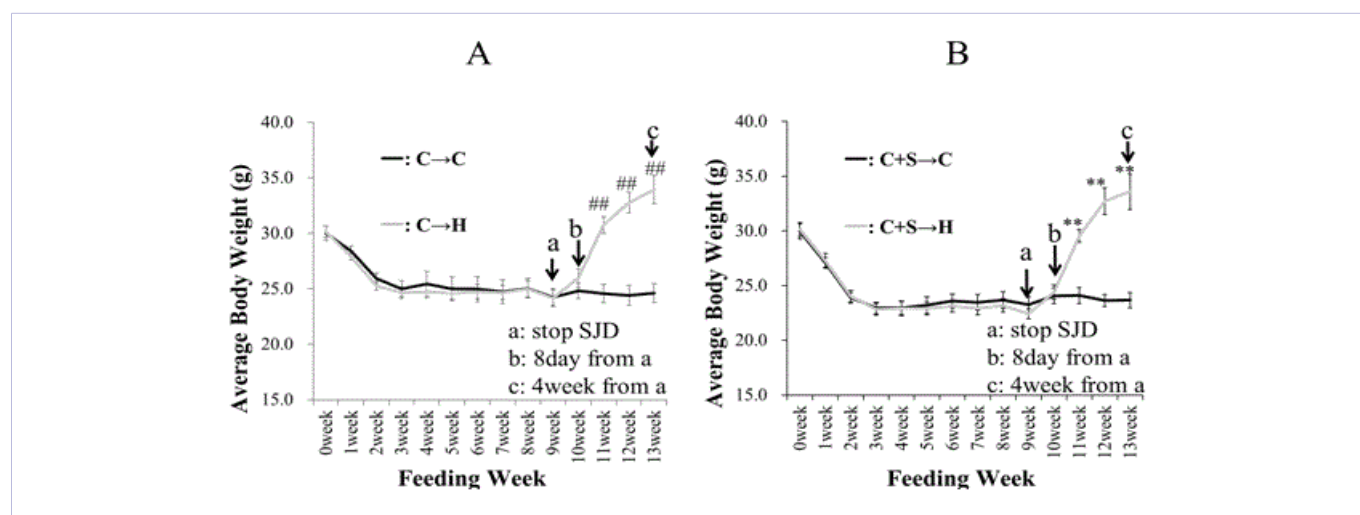


Figure 2A & 2B: Effects of SJD on the changes in body weight

Each value shows the mean $\pm$ S.E. ##; compared with ⑤C→C group in each week ($p < 0.01$). **; compared with ⑦C+S→C group in each week ($p < 0.01$)

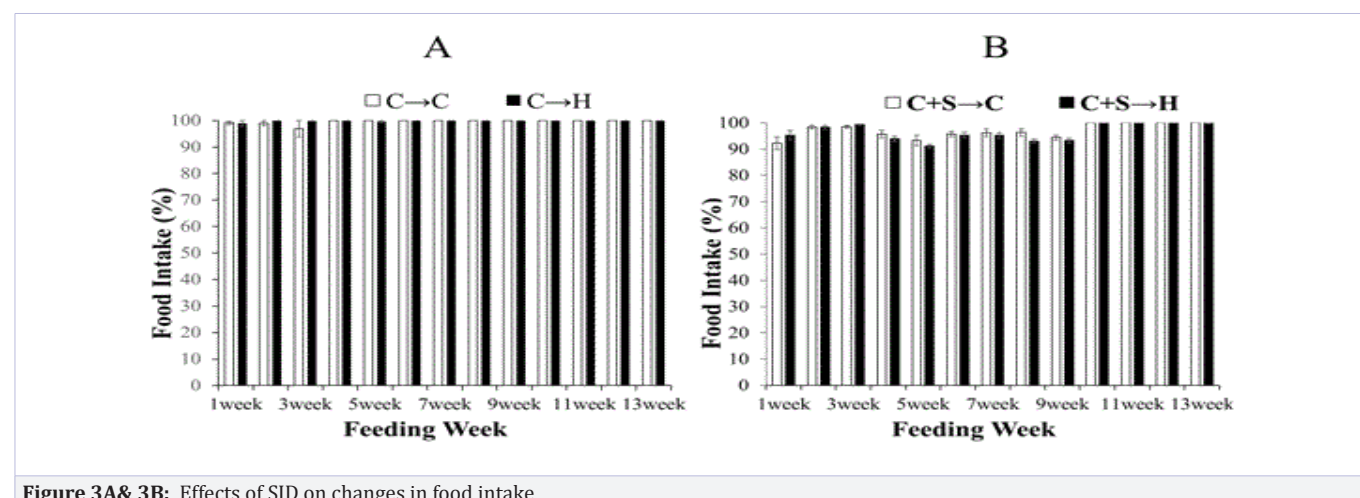


Figure 3A & 3B: Effects of SJD on changes in food intake

groups ⑦C+S→C and ⑧C+S→H are demonstrated in Figure 2B.

The group ⑥C→H showed a significant increase in body weight after the 11th week onward compared group ⑤C→C ($p < 0.01$). In addition, group ⑧C+S→H showed significant weight gain from the 11th week in comparison to group ⑦C+S→C ($p < 0.01$). The groups ⑤C→C and ⑦C+S→C did not show any change in their body weights.

Effects of SJD on Changes in Food Intake

Figure 3A, 3B demonstrated the results of changes in food intake in groups ⑤C→C and ⑥C→H, and groups ⑦C+S→C and ⑧C+S→H, respectively. These results suggest that all mice in these four groups ate more than 90% of the feed.

Adiponectin Levels After 8 Weeks

Of note, no differences in food intake were observed in groups ①C and ②C+S (data not shown).

The RT-PCR measurements of mRNA expression levels of adiponectin in adipose tissue at the end of the experiment are shown in Figure 4. Gene expression profiles were normalized to the expression levels of GAPDH and are represented as a ratio to group ①C set at 1 as a control. The GAPDH ratio of group ②C+S was 1.66 ± 0.14 . This ratio was significantly higher than that of group ①C ($p < 0.01$) Figure 4.

These results suggest that the increase of adiponectin can be induced by SJD treatment in the normal condition.

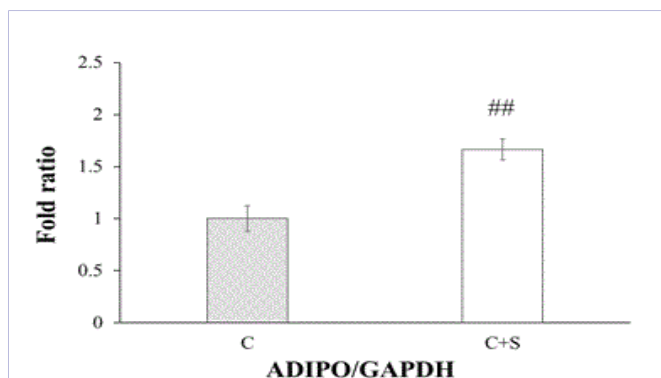


Figure 4: Adiponectin levels after 8 weeks Each value shows the mean±S.E. #, compared with ①C group ($p < 0.05$)

Effect of the SJD on the Weight of Visceral Fat Mass

Figure 5 shows the changes in total visceral fat (peri-intestinal fat, peri-testicular fat, and peri-renal fat) in groups ①C and ②C+S Figure 5A, in groups ③C+S→C8d and ④C+S→H8d Figure 5B and in groups ⑤C→C, ⑥C→H, ⑦C+S→C and ⑧C+S→H Figure 5C. Visceral fat volumes at the end of the test were 2.9 ± 0.1 g in group ①C, 2.6 ± 0.1 g in group ②C+S, 2.8 ± 0.1 g in group ③C+S→C8d, 9.3 ± 1.3 g in group ④C+S→H8d (dissected at the 8th day), 2.7 ± 0.2 g in group ⑤C→C, 8.8 ± 1.9 g in group ⑥C→C (dissected at the 28th day), 2.6 ± 0.2 g in group ⑦C+S→C, and 9.0 ± 2.8 g in group ⑧C+S→H (dissected at the 28th day). Groups

③C+S→C8d and ⑦C+S→C, which received the CE-2, did not show any significant changes in comparison to the CE-2 fed group ⑤C→C. Thus, there was no significant difference between group ⑥C→H and group ⑧C+S→H. The continuous feeding of SJD is essential to inhibit the increase of visceral fat mass.

Effect of SJD on the Plasma Lipid Metabolic Parameters

The effects on total plasma cholesterol Figure 6 and TG Figure 7 levels were detected.

Figure 6 represents plasma cholesterol levels in groups ①C and ②C+S Figure 6A, in groups ③C+S→C8d and ④C+S→H8d Figure 6B and in groups ⑤C→C, ⑥C→H, ⑦C+S→C and ⑧C+S→H Figure 6C. Plasma total cholesterol levels were 91.9 ± 26.0 mg/dL in group ①C, 53.0 ± 9.5 mg/dL in group ②C+S, 48.3 ± 17.0 mg/dL in group ③C+S→C8d, 99.7 ± 4.5 mg/dL in group ④C+S→H8d, 59.3 ± 11.4 mg/dL in group ⑤C→C, 109.3 ± 25.4 mg/dL in group ⑥C→H, 40.4 ± 11.3 mg/dL in group ⑦C+S→C, and 127.9 ± 20.3 mg/dL in group ⑧C+S→H. The CE-2 with the SJD fed groups (③C+S→C8d and ⑦C+S→C) did not show any significant changes compared to the CE-2 alone fed group (⑤C→C). There was no significant difference between group ⑥C→H and group ⑧C+S→H.

Figure 7 shows the plasma TG levels in groups ①C and ②C+S Figure 7A, groups ③C+S→C8d and ④C+S→H8d Figure 7B and groups ⑤C→C, ⑥C→H, ⑦C+S→C and ⑧C+S→H Figure 7C. Total plasma triglyceride levels were 11.4 ± 12.9 mg/dL in group ①C, 22.6 ± 13.1 mg/dL in group ②C+S, 4.5 ± 10.0 mg/dL in group ③C+S→C8d, 55.3 ± 3.6 mg/dL in group ④C+S→H8d, 4.3 ± 5.5 mg/dL in group ⑤C→C, 47.3 ± 6.5 mg/dL in group ⑥C→H, 5.5 ± 10.3 mg/dL in group ⑦C+S→C, and 54.3 ± 18.9 mg/dL in group ⑧C+S→H. The CE-2 with the SJD fed groups (③C+S→C8d and ⑦C+S→C) did not show any significant changes in comparison with the CE-2 alone fed group (⑤C→C). There was no significant difference between groups ⑥C→H and ⑧C+S→H.

These results suggest that the continuous feeding of SJD suppresses the increase in cholesterol and TG levels. However, abrogation of the continuous SJD feeding leads to the concomitant increase of these levels.

Effect of SJD on Mrna Expression Levels of the Adipocytokines

The mRNA expression levels of adiponectin and leptin in adipose tissue were measured using the RT-PCR method at the end of the experiment. The gene expression was normalized using expression levels of GAPDH and finally represented as fold changes in comparison to group ①C set as 1. Figure 8 represents adiponectin levels in groups ①C and ②C+S Figure 8A, in groups ③C+S→C8d and ④C+S→H8d Figure 8B and in groups ⑤C→C, ⑥C→H, ⑦C+S→C and ⑧C+S→H Figure 8C. The expression ratios of adiponectin were 1.7 ± 0.1 in group ②C+S, 1.6 ± 0.7 in group ③C+S→C8d, 0.43 ± 0.1 in group ④C+S→H8d, 0.9 ± 0.1 in group ⑤C→C, 0.4 ± 0.1 in group ⑥C→H, 1.2 ± 0.1 in group ⑦C+S→C, and 0.5 ± 0.1 in group ⑧C+S→H. In comparison to group ⑤C→C,

significant increases were observed in groups ③C+S→C8d ($P < 0.01$, respectively). Furthermore, a significant decrease was observed in group ⑦C+S→C, compared to group ③C+S→C8d ($P < 0.01$). These results further confirmed that adiponectin levels decreased unless the SJD was continuously consumed.

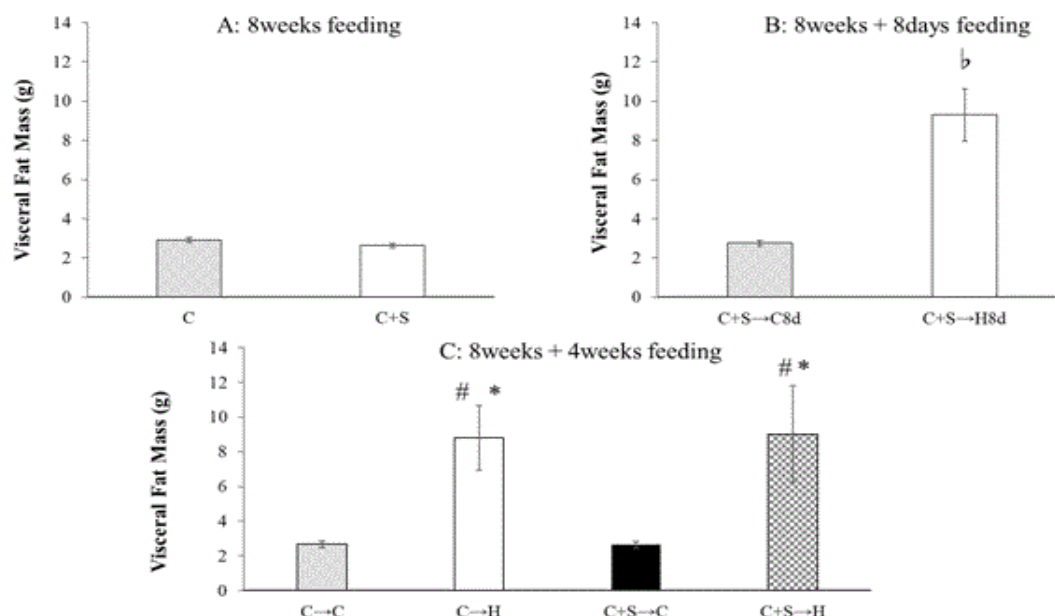


Figure 5A, 5B& 5C: Effects of SJD on the weight of visceral fat mass

Each value shows the mean±S.E. b; compared with ③ C+S→C8d group in each group ($p < 0.05$) #; compared with ⑤ C→C group in each group ($p < 0.05$) *; compared with ⑦ C+S→C group in each group ($p < 0.05$)

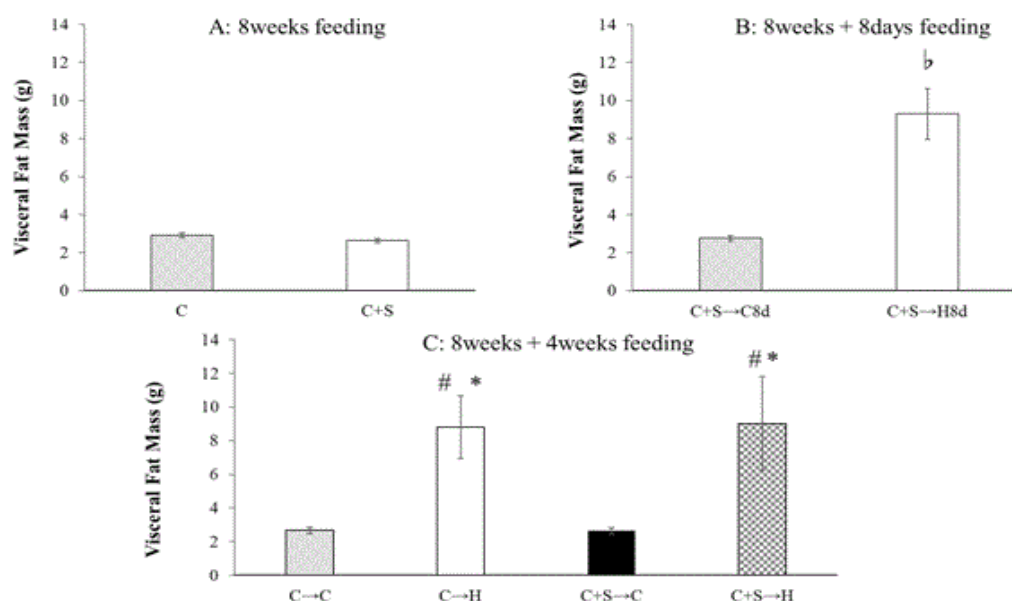


Figure 6A, 6B& 6C: Effect of SJD on the Plasma lipid metabolic

parameters Each value shows the mean±S.E. *; compared with ⑦ C+S→C group in each group ($p < 0.05$)

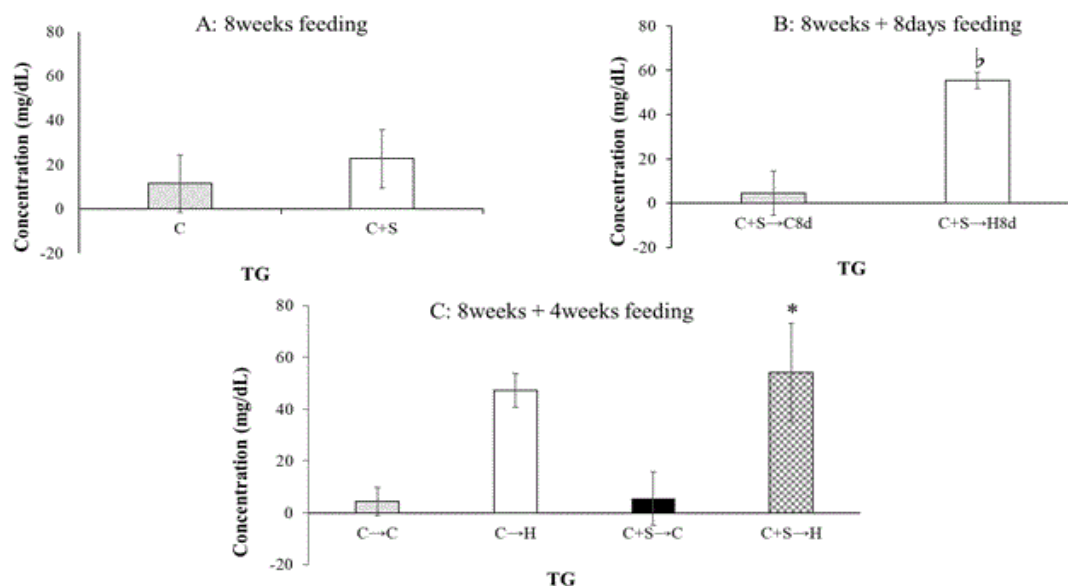


Figure 7A, 7B & 7C: Effect of SJD on the Plasma lipid metabolic parameters
Each value shows the mean±S.E. ^{*}; compared with ⑦C+S→C group in each group (p<0.05)

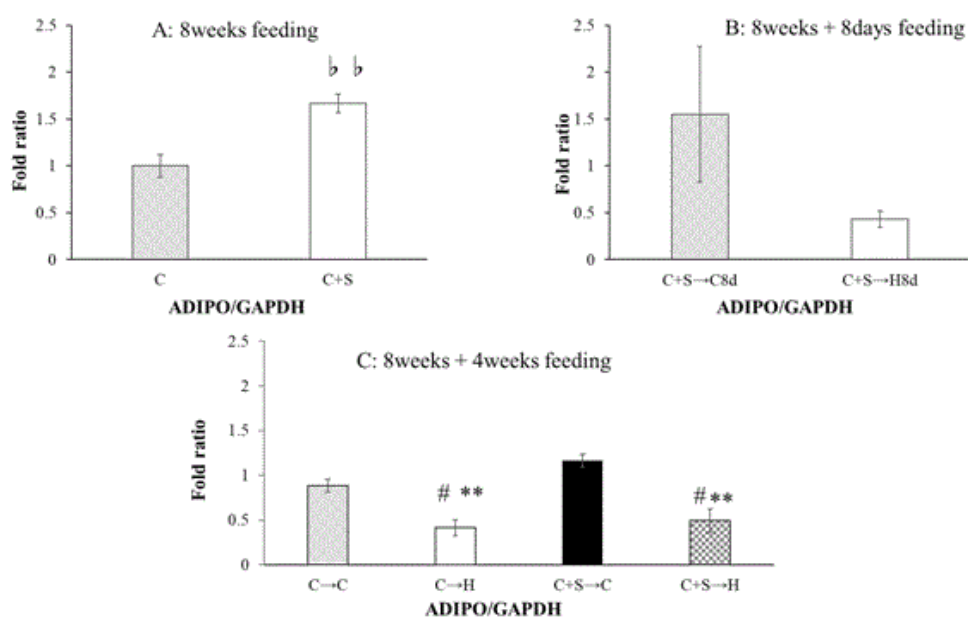
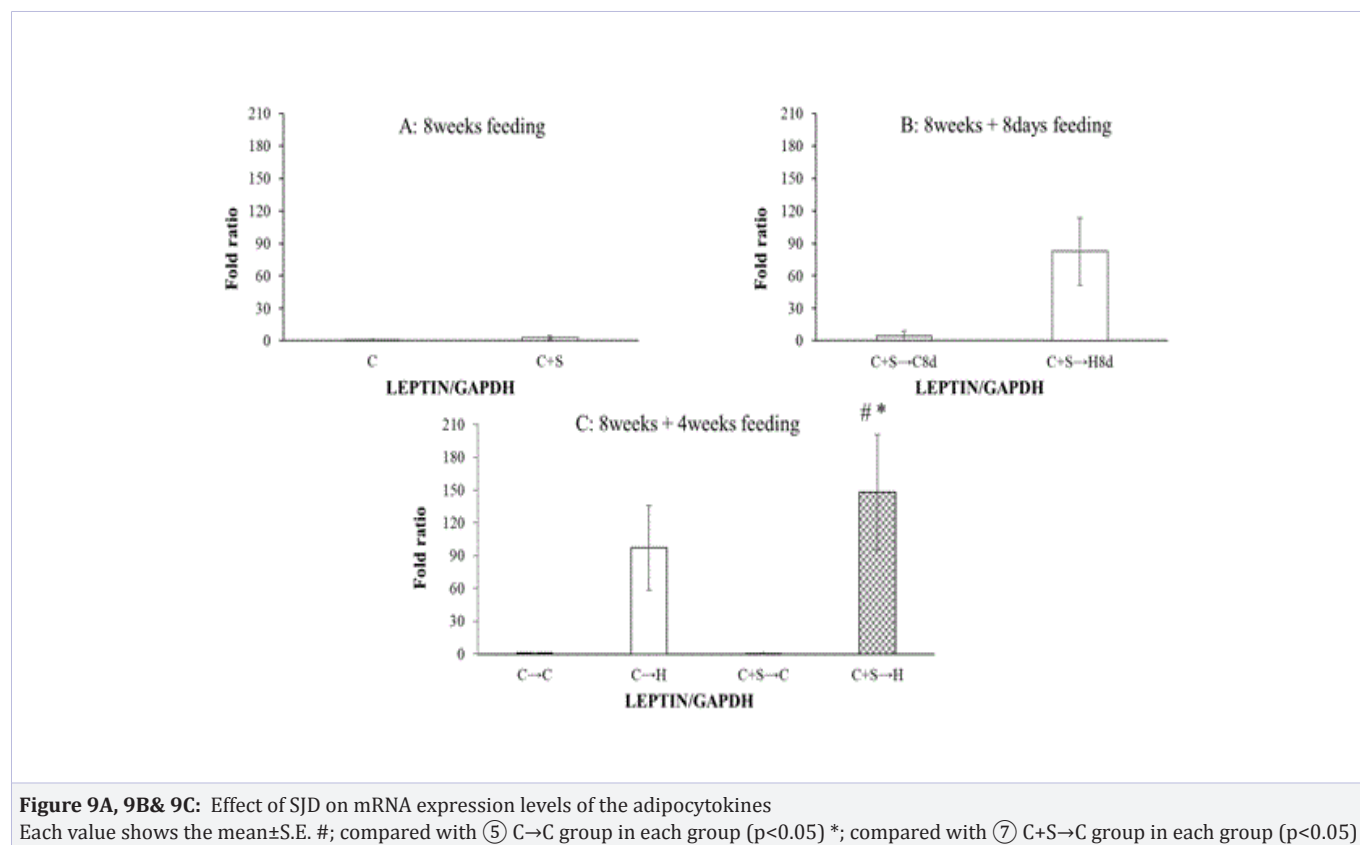


Figure 8A, 8B & 8C: Effect of SJD on mRNA expression levels of the adipocytokines
Each value shows the mean±S.E. ^{bb}; compared with ① C group in each group (p<0.01) [#]; compared with ⑤ C→C group in each group (p<0.05) ^{**}; compared with ⑦ C+S→C group in each group (p<0.01)

Figure 9 represents leptin levels in groups ①C and ②C+S Figure 9A, in groups ③C+S→C8d and ④C+S→H8d Figure 9B and in groups ⑤C→C, ⑥C→H, ⑦C+S→C and ⑧C+S→H Figure 9C. The expression ratios of leptin were 2.9 ± 1.4 in group ②C+S, 4.7 ± 4.3 in group ③C+S→C8d, 82.5 ± 30.7 in group ④C+S→H8d, 0.7 ± 0.1 in group ⑤C→C, 97.2 ± 38.8 in group ⑥C→H, 0.9 ± 0.1 in group ⑦C+S→C and 148.0 ± 52.9 in group ⑧C+S→H. The CE-2 with the SJD fed groups (②C+S, ③C+S→C8d, and ⑦C+S→C) did not show any significant changes, compared to the CE-2 alone fed group (⑤C→C). In cases of the HFD groups, the expression ratios of leptin were and there was no significant difference between group ⑥C→H and group ⑧C+S→H.



Effect of SJD on Plasma Leptin Expression Levels

The effects of SJD on plasma leptin levels are shown in groups ①C and ②C+S Figure 10A, in groups ③C+S→C8d and ④C+S→H8d Figure 10B and in groups ⑤C→C, ⑥C→H, ⑦C+S→C and ⑧C+S→H Figure 10C. Total plasma leptin levels were 166.6 ± 24.6 mg/dL in group ①C, 178.9 ± 22.0 mg/dL in group ②C+S, 256.4 ± 70.0 mg/dL in group ③C+S→C8d, 523.3 ± 73.4 mg/dL in group ④C+S→H8d, 52.4 ± 32.0 mg/dL in group ⑤C→C, 65.2 ± 32.1 mg/dL in group ⑥C→H, 27.5 ± 30.1 mg/dL in group ⑦C+S→C and 117.5 ± 38.5 mg/dL in group ⑧C+S→H. When compared to group ⑤C→C, a significant increase was observed in group ③C+S→C8d ($P < 0.05$). In addition, compared to group ⑥C→H group ④C+S→H8d showed a significant increase in total plasma leptin levels ($P < 0.01$).

Discussion

In this study, we investigated changes in adiponectin and leptin levels by daily ingestion of the SJD in normal mice. After adherence to the SJD, body weight, visceral fat, blood cholesterol, blood TG, blood leptin levels, and adiponectin/leptin levels in

adipose tissue were measured during the HFD.

According to the experimental design, normal mice continuously consumed the SJD for two months, and an increase in adiponectin levels in adipose tissue was confirmed. Afterward, the SJD was abrogated, and either the CE-2 or HFD were continuously given for consumption for 4 weeks. Then the body weight and visceral fat volume were measured. The detection of blood cholesterol, TG, and plasma leptin levels was performed using biochemical methods, whereas adiponectin/leptin levels in adipose tissue were measured by RT-PCR. Since consumption of food occurred via free administration, the corresponding feeding amount was set at approximately 70% of 4.5 g, which is the average daily food intake amount for normal mice. Moreover, each mouse was kept as individual breeding. Then we checked the amount of food consumed every morning Figure 3A & 3B.

In our study, it was confirmed that adiponectin increased by continuous ingestion of the SJD in normal mice Figure 4&8, and this result is in accordance with results from our previous study, which also showed an increase in adiponectin [2]. Fucoxanthin, which is contained in brown algae, such as wakame and root kelp,

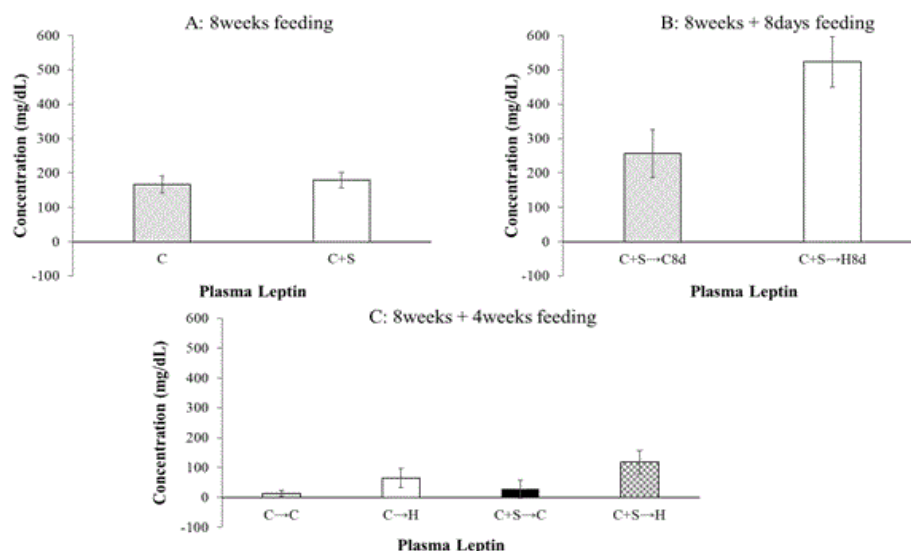


Figure 10A, 10B& 10C: Effect of SJD on mRNA expression levels of the adipocytokines

promotes energy consumption by increasing the concentration of uncoupling protein-1 (UCP1) in brown adipose tissue (BAT) and increases adiponectin levels, leading to the anti-obesity effects [5]. This finding suggests that fucoxanthin is also involved in adiponectin's increase during the SJD.

Adiponectin is one of adipocytokines of adipose tissue which is secreted from the adipocytes of normal size. It is considered as the most abundant gene product in adipose tissue and accounts for 0.01% of total plasma protein [6,7]. Adiponectin is essential for stimulating fatty acid oxidation and regulating glucose and lipid metabolism in almost all major target tissues [8,9]. In addition, adiponectin levels in adipose tissue decrease due to weight gain and obesity. It is reported that this observation is related to the development of insulin resistance [10,11].

In this study, the significant increase of adiponectin levels in the adipose tissue was confirmed at the 8th week in group ②C+S compared to group ①C Figure 4. In the CE-2-fed groups, after changing to the SJD, adiponectin levels in the adipose tissue decreased at the 8th day and continued to decrease up into the 4th week Figure 8. Furthermore, there was no increase in body weight or visceral fat Figure 2&5. These results suggest that a decrease of adiponectin levels in adipose tissue does not relate to obesity. However, it was recognized that adiponectin levels are increased by daily consumption of the SJD, and discontinuing SJD ingestion gradually induces a decrease in adiponectin levels. These results suggest that the continuous ingestion of the SJD can prevent type 2 diabetes and certain cardiovascular diseases, which are caused by the decrease of adiponectin [12-15].

It has a significant effect on the increase and decrease in visceral fat and has been reported to be correlated with ingestion and weight gain [16,17]. It also stimulates the increased consumption

of energy, such as suppression of food intake and lipolysis by activating the leptin receptor expressed in neurons of the hypothalamus [18]. In the HFD group, we observed that the increased levels of leptin in adipose tissue did not show any protection against obesity [1,2]. These results suggest that the leptin regulation in the HFD group is dysregulated by cellular transduction of the JAK2-STAT3 signaling pathway and the desensitization of leptin receptors [19,20]. However, treatment with the SJD showed the suppression of obesity due to the improvement in leptin resistance [1,2]. In this study, in order to clarify the significance of continuous SJD treatment, mice adhered to the SJD for 8 weeks, followed by its abrogation. In such conditions, leptin resistance was confirmed on the eighth day after intake cancellation. However, four weeks later, leptin resistance and weight gain in these mice were confirmed. These results indicate that the cessation of continuous ingestion of the SJD caused obesity by inducing relapse of leptin resistance in adipocytes Figure 9&10.

Thus, it was confirmed that adiponectin was increased by consuming the SJD. However, after SJD abrogation, the animals of the group that switched to the HFD developed increased body weight, visceral fat, cholesterol, and TG levels. Therefore, it was confirmed that the effect of increasing adiponectin by SJD could be ignored unless administration is continuous.

When the SJD was taken for 8 weeks, no significant changes were observed in adipose tissue and plasma leptin Figure 9&10. However, on day 8, after the SJD cancellation and switching to the HFD, increases in adipocyte and plasma leptin levels were confirmed Figure 9&10. After 4 weeks, adipose tissue leptin levels increased, while plasma leptin concentration levels decreased. Moreover, significant increases in body weight and visceral fat

were observed. Interestingly, when continuous administration of the SJD in combination with the HFD was performed, the inhibition of leptin resistance in adipose tissue was observed [1,2]. Taken together, it was revealed that the SJD did not affect leptin kinetics during continuous ingestion in the normal state. However, the anti-obesity effect of the SJD during obesity is due to the increased plasma leptin levels and inhibition of leptin resistance in adipose tissue.

In our study, the effect of continuous ingestion of the SJD on increasing fat adiponectin levels in normal mice was further confirmed. Moreover, anti-obesity effects due to adiponectin were not observed. However, it has been suggested that it may prevent type 2 diabetes and certain cardiovascular diseases, which are caused by adiponectin reduction in mice [21,22].

The anti-obesity mechanism of the continuous ingestion of the SJD can be explained by three mechanisms. First, the increase of adiponectin levels due to the promotion of energy consumption by increasing the concentration of UCP1 in BAT [5]. Next, the inhibition of leptin resistance due to the control of cellular transduction via the JAK2-STAT3 signaling pathway and accompanied desensitization of leptin receptors in comparison between normal and obesity stages [19,20]. Based on the data, it can be recognized that continuous uptake of the SJD every day without interruption has an essential beneficial impact on health.

Conclusion

Herein we reproduced and confirmed data showing an increase in adiponectin levels induced by continuous ingestion of the SJD in non-obese mice. This study demonstrated that the increase in adiponectin did not influence anti-obesity effects. However, it can be suggested that SJD adherence can prevent type 2 diabetes and certain cardiovascular diseases, which are caused by adiponectin reduction.

Concerning the leptin regulation by the SJD treatment, we revealed that the consequent SJD feeding induced the protection of leptin resistance in the adipose tissue and increased the plasma leptin levels. However, these regulations can be disturbed by discontinuing SJD feeding. The daily uptake of the SJD is vital for maintaining an overall healthy condition.

Authors Contributions

YH mainly performed most of the experiments; ST, SA, RO, and TS partially helped in the performance of the important parts of the experiments; NI and YW provided helpful advice during the research; YW designed the experiments; KT provided critical suggestions on the writing. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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