Improvement Effect of Sweet Basil (Ocimum basilicum L.) Powder Intake on Obese Mice Fed a High-fat and High-sucrose Diet

Chikako Kiyose¹,²*, Haruka Takeuchi¹, Yoshimni Yabe¹, Tomoya Koike², Kazutaka Sakiya², Mana Nagase², Rieko Tanaka-Yachi³, and Chie Takahashi-Muto⁴

¹ Department of Applied Chemistry and Bioscience, Graduate School of Engineering, Kanagawa Institute of Technology, 1030 Shimo-ogino, Atsugi-shi, Kanagawa 243-0292, JAPAN
² Department of Nutrition and Life Science, Kanagawa Institute of Technology, 1030 Shimo-ogino, Atsugi-shi, Kanagawa 243-0292, JAPAN
³ Department of Pharmacology, National Research Institute for Child Health and Development, Tokyo 157-8535, JAPAN
⁴ Department of Clinical Nutrition, Kitasato Junior College of Health and Hygienic Sciences, Niigata 949-7241, JAPAN

Abstract: This study aimed to determine if there are anti-inflammatory and anti-obesity effects of sweet basil, an herb, in mice. Sweet basil was administered as a powder to male C57BL/6Jcl mice, which were divided into three groups: the (control [C], high-fat and high-sucrose diet [H], and high-fat and high-sucrose diet plus sweet basil powder [HB]) groups. The mice were fed for 12 weeks and the dry sweet basil powder comprised 1% per kg of the diet. From experiment third week, the average body weight was significantly higher in the H group than in the C group. The average body weight was significantly lower in the HB group than in the H group, but food intake did not significantly differ between the H and HB groups. Liver weight was drastically lower in the HB group than in the H group. Perirenal fat weight and epididymal fat weight were not significantly different between the H and HB groups. Therefore, we assumed that body-weight reduction caused by sweet basil powder intake depended on inhibition of liver enlargement. We then examined lipid metabolism-related gene expression in the mice livers. Expression of the sterol response element binding protein 1-c gene tended to be lower in the HB group than in the H group (p=0.056). We speculated that sweet basil inhibited liver enlargement by suppressing fatty acid synthesis. Moreover, expression of the monocyte chemoattractant protein-1 gene in epididymal fat was significantly lower in the HB group than in the H group. Sweet basil powder appears to have a potent anti-inflammatory effect in the adipose tissue of mice fed a high-fat and high-sucrose diet.

Key words: sweet basil, anti-obesity, anti-inflammation, in vivo, a high-fat and high-sucrose diet

1 Introduction

In Japan, one of three adult males is obese, which persists long term. Obesity is associated with not only higher body weight, but also excessive fat accumulation. The definition of obesity is a body mass index (BMI) > 25 kg/m² in Japan. In a prospective cohort study, Ishikawa–Tanaka et al.¹ investigated the effects of the degree of BMI and weight gain as risks for hypertension, hypercholesterolemia, and diabetes in 4373 Japanese people. Those researchers found that the risks greatly increased in subjects with a BMI > 27 kg/m² for hypertension and > 29 kg/m² for diabetes and hypercholesterolemia. Additionally, Aoyagi et al.² reported that a BMI > 25 kg/m² and a waist circumference > 85 cm for males and 90 cm for females tended to be associated with diabetes and was significantly associated with dyslipidemia. Therefore, obesity was shown to be a risk factor for onset of those lifestyle-related disease. Why is obesity a risk factor for onset of dyslipidemia? Bastard et al.³ reported that obesity accelerates production of inflammatory instruction factors, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6, which is related to onset of lifestyle-related disease and insulin resistance. The cause is that expression of monocyte chemoattractant protein-1 (MCP-1) increases in enlarged mesentery adipose tissue, and then monocytes infiltrate adipose tissue where they become macrophages⁴. Furthermore, the macro-
phages within adipose tissue produce inflammatory factors, such as TNF-α and nitric oxide, and an inflammatory reaction in the adipose tissue further aggravates inflammation. It is speculated that accentuation of such chronic inflammation in adipose tissue triggers the lifestyle-related disease.

Sweet basil (Ocimum basilicum L.) is a well-known herb that belongs to the Lamiaceae family and has a sweet taste and refreshing fragrance. This herb is often used in Italian cooking. Reportedly, the essential oil and extract of sweet basil have antioxidant and antibacterial activities. We previously reported that the methanol extract of sweet basil had an anti-inflammatory effect on 3T3-L1 adipocyte-induced inflammation. A co-culture of 3T3-L1 adipocytes and RAW264.7 macrophages is widely used as an inflammatory instruction model in adipose tissue. We found that a methanol extraction of sweet basil significantly inhibited the expressions of the Il-1β, Il-6, and MCP-1 genes in 3T3-L1 adipocytes in which the gene expressions of inflammatory cytokines had increased. Therefore, we speculated that sweet basil should show in vitro anti-inflammatory activity in adipocytes. The study aim was to determine what kind of effect a sweet basil powder intake shows to the obesity-model mice fed a high-fat and high-sucrose diet in vivo.

2 Experimental Procedures
2.1 Materials
Sweet basil was purchased from S&B Food Inc. (Tokyo, Japan). Fresh sweet basil leaves were freeze-dried, powdered, and then stored at −80°C.

2.2 Experimental procedure
All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals at the Kanagawa Institute of Technology. We used male C57BL/6Jcl mice (3 weeks old, n = 19), which were purchased from CLEA Japan, Inc. These mice were housed individually in plastic cages and kept in an environment controlled at 23°C ± 2°C and 55% ± 5% humidity, with a 12 h/12 h light/dark cycle. The mice were initially fed a control diet for 1 week to allow them to adapt to the new environment. Thereafter, they were divided according to their average weight to avoid differences into three groups: the control (C, n = 7), high-fat and high-sucrose (H, n = 6), and high-fat and high-sucrose diet plus sweet basil dried powder (HB, n = 6) groups. Table 1 presents the diet composition of each group. The feed and water were supplied ad libitum for 12 weeks. After a 16-h fast, all of the mice were sacrificed under isoflurane anesthesia, and the arterial blood and each tissue were extracted for the analysis.

2.3 Measurement of triglyceride (TG) and glucose concentration in mice serum
The concentration of TG in mice serum was measured by using a triglyceride E-test kit (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan). Additionally, the concentration of glucose in mice serum was measured by using a glucose C-test kit (FUJIFILM Wako Pure Chemical Corp.).

| Group               | C   | H   | HB  |
|---------------------|-----|-----|-----|
| Comstarch           | 400 | 84  | 74  |
| Milk-casein         | 200 | 200 | 200 |
| α-Comstarch         | 132 | 28  | 28  |
| Sucrose             | 100 | 312 | 312 |
| Lard                | 0   | 208 | 208 |
| Soybean oil         | 70  | 70  | 70  |
| Cellulose           | 50  | 50  | 50  |
| Mineral mix(AIN-93G)| 35  | 35  | 35  |
| Vitamin mix         | 10  | 10  | 10  |
| l-Cystine           | 3   | 3   | 3   |
| l-Buthylhydroquinone| 0.014| 0.014| 0.014|
| Sweet basil powder  | 0   | 0   | 10  |
| Total (g)           | 1000.014| 1000.014| 1000.014|
| Total energy(kcal/kg diet) | 3958 | 4998 | 4998 |
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2.4 mRNA analysis of mice liver and adipose tissue
Total RNA was extracted by using Sepasol®-RNA I Super G solution. The amount and purity of the RNA were measured at 260 and 280 nm with a NanoDrop Q5000 spectrophotometer (Tommy Seiko Co., Ltd., Tokyo, Japan). The total RNA was reverse transcribed into cDNA by using a High-Capacity RNA-to-cDNA Kit. The mRNA expression of each gene was measured by using a 7500 Fast Real-Time PCR system and Taqman® Gene Expression Assays (Applied Biosystems, Thermo Fisher Scientific K.K., Tokyo, Japan). GAPDH, a housekeeping gene was used as the reference. The Assay IDs and RefSeqs of the mouse primers used for the quantitative real-time PCR are shown in Table 2. The differences between the Ct values of the samples and GAPDH were calculated and the logarithm of this difference was taken as the measured value for each sample.

2.5 Statistical analysis
The data are presented as mean ± SDs. Differences were evaluated by using one-way ANOVA followed by Tukey’s HSD post-hoc test and were considered statistically significant at p<0.05. The analysis was performed by using IBS SPSS Statistics 21 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Body-weight gain of each group during the experimental period
Figure 1 shows the body-weight gain of each group during the experimental period (12 weeks). The average body weight was significantly lower in the HB group than in the H group in the third week of the experiment, and the difference continued until the end of the experiment.

3.2 Final body weights, food intakes, energy intakes, and tissue weights in each group (Table 3)
The final body weight was significantly higher in the H group than in the C group and was drastically lower in the HB group than in the H group. On the other hand, food intake was significantly lower in the H and HB groups than in the C group, but did not significantly differ between the H and HB groups. Therefore, we found that the body-weight loss caused by intake of sweet basil powder did not appear to depend on the food intake. The liver weight was drastically higher in the H group than in the C group. Additionally, the liver weight was significantly lower in the HB group than in the H group. The adipose tissue weight (peri-renal fat and epididymal fat) was significantly higher in the H group than in the C group. However, there were no significant differences between the H and HB groups. Therefore, there was not an anti-obesity effect by sweet basil powder intake in this study. We concluded that the suppression of weight gain caused by intake of basil powder depended on depression of the liver enlargement caused by a high-fat and high-sucrose diet.

3.3 Concentrations of TG and glucose in mice plasma
The concentration of glucose in the H group was significantly higher than in the C group, however, there was no significant difference between the H and HB groups. There were no significant differences in the TG concentrations among the C, H, and HB groups (Table 4). Therefore, we assumed that basil powder intake did not affect lipid transportation in the blood stream.

Table 2 Primer probe mixture of each gene and GAPDH.

| Gene   | AssayID      | RefSeq         |
|--------|--------------|----------------|
| Srebp  | Mm01231183_m1| NM_013495.2    |
| Cpt1   | Mm00550338_m1| NM_011480.3    |
| Ccl2   | Mm00441242_m1| NM_011333.3    |
| Il1b   | Mm00434228_ml| NM_008361.3    |
| GAPDH  | Mm99999915_g1| NM_008084.2    |

Assay ID and reference sequence number (RefSeq) of primer probe mixtures used in TaqMan® Gene Expression Assays (Applied Biosystems).

Fig. 1 Body-weight gain of each group for 3 months. The data are presented as mean ± SDs (n = 6–7). Different superscript letters indicate significant differences by two-way ANOVA, followed by Tukey’s HSD post-hoc test (p<0.05).
3.4 Gene expressions of the Srebp, Cpt1, Il1b, and Ccl2 in mice livers (Fig. 2)

Since basil powder intake inhibited mice liver enlargement caused by a high-fat and high-sucrose diet, we next measured two factors that affect lipid metabolism in the liver: expression of the sterol response element binding protein 1-c (Srebp) gene and of the carnitine palmitoyl-transferase 1 (Cpt1) gene. The expression of Srebp, a factor in lipid synthesis, was much higher in the H group than in the C group. This finding implies that lipid synthesis in the livers of mice fed a high-fat and high-sucrose diet was activated. However, it was clear that basil powder intake tended to inhibit the lipid synthesis (p = 0.056) (Fig. 2A). On the other hand, the expressions of the Cpt1 gene, a lipid catabolism factor, were markedly lower in the H and HB groups than in the C group, but there were no significant differences in the gene expressions between the H and HB groups (Fig. 2B). Therefore, we speculated that sweet basil added to the diet inhibited lipid synthesis in the livers of mice, which may have then suppressed the enlargement of the livers in the mice. Moreover, we measured two factors that affect inflammation in the liver of mice fed a high-fat and high-sucrose diet: expression of the interleukin-1β (Il1b), inflammatory cytokine and Ccl2, inflammatory chemokine. There were no significant differences in the expression of Il1b gene of mice livers among three groups (Fig. 2C). On the other hand, the expression of Ccl2 gene in the H group was significantly higher than that in the C group but there were no significant differences in the gene expressions between H group and HB group (Fig. 2D). Accordingly, we assumed that the livers of mice fed a high-fat and high-sucrose diet begin to receive the dysfunction, but there was not the improvement effect due to sweet basil powder intake.

3.5 Gene expressions of Il1b and Ccl2 in mice perirenal fat

It is known that the adipose tissue of mice fed a high-fat and high-sucrose diet long term induces inflammation. Thus, we measured the expressions of the Il1b gene, an inflammatory cytokine, and of the Ccl2 (MCP-1) gene, a chemokine, in the perirenal fat of mice (Fig. 3). The expressions of the Il1b gene were not significantly different among the C, H, and HB groups (Fig. 3A). On the other hand, the expression of the Ccl2 gene was significantly higher in the H group than in the C group. However, the expression of the Ccl2 gene was drastically lower in the HB group than in the H group (Fig. 3B). These results suggest-

Table 3  Final body weight, Food intake and each tissue weight.

|                     | C       | H       | HB      |
|---------------------|---------|---------|---------|
| Final body weight (g)| 32.0±2.3a | 44.4±1.5b | 40.1±2.6a |
| Food intake (g/day)  | 3.5±0.5b | 2.9±0.1b | 2.7±0.2b |
| Energy intake (kcal/day) | 14.0±1.7 | 14.2±0.5 | 13.7±0.8 |
| Liver (g)            | 1.1±0.1a | 1.4±0.2b | 1.2±0.1a |
| Liver (g/100g body weight) | 3.4±0.2a | 3.2±0.3b | 3.0±0.1b |
| Perirenal fat (g)    | 0.4±0.1a | 1.1±0.1b | 0.9±0.3b |
| Perirenal fat (g/100g body weight) | 1.3±0.2a | 2.4±0.2b | 2.3±0.7b |
| Epididymal fat (g)   | 1.1±0.3b | 2.5±0.2b | 2.2±0.3b |
| Epididymal fat (g/100g body weight) | 3.3±0.8a | 5.7±0.5b | 5.4±0.4b |

1 Values are mean ± SDs, n=6-7
2 Different superscript letters are significantly difference by using one-way ANOVA followed by Turkey’s HSD post-hoc test (p<0.05)

Table 4  Glucose and TG concentration in mice plasma.

|                   | C       | H       | HB      |
|-------------------|---------|---------|---------|
| Glucose (mg/mL)   | 163.6±19.1b | 227.6±32.0b | 195.3±44.9b |
| TG                | 104.5±50.7 | 78.2±9.5 | 93.7±24.2 |

1 Values are mean ± SDs, n=5-7
2 Different superscript letters are significantly difference by using one-way ANOVA followed by Turkey’s HSD post-hoc test (p<0.05)
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Discussion

In this study, we determined if there were anti-obesity and anti-inflammatory effects of sweet basil powder ingested by mice fed a high-fat and high-sucrose diet. The final body weight was significantly lower in the HB group than in the H group for 3 months. However, no significant differences in dietary and energy intake were found between the H and HB groups (Table 3). Hence, we assumed that the body-weight reduction depended on the basil powder intake, a finding that has not been previously reported and is considered to be novel. Unluckily, there were not significant differences in the adipose tissue weights between H group and HB group. Therefore, this result did not show that the sweet basil powder intake has an “anti-obesity” effect in this study.

In the case of ingestion of vegetable food powder, such as sweet basil, the influence of dietary fiber should be considered. In this study, we presumed that the influence of dietary fiber in sweet basil on lipid absorption may be low because the amount of dietary fiber in sweet basil is less than the amount of cellulose added experimental diet. However, we didn’t determine the amount of dietary fiber in sweet basil. Hence, we are likely to consider the dietary fiber in sweet basil in the next experiment. On the other hand, Harach et al.11 reported that treatment of mice with

Fig. 2  Effects of sweet basil powder intake on lipid metabolism-related gene expression in the livers of mice fed a high-fat and high-sucrose diet for 12 weeks. (A) Srebp; (B) Cpt1, (C) Il1b, (D) Ccl2. The data are presented as mean ± SDs (n = 6–7). Statistical analysis was performed by two-way ANOVA, followed by Tukey’s HSD post-hoc test (*p < 0.05, **p < 0.01).

Fig. 3  Effects of sweet basil powder intake on expressions of inflammatory cytokine genes in the perirenal fat of mice fed a high-fat and high-sucrose diet for 12 weeks. (A) Il1b; (B) Ccl2. The data are presented as mean ± SDs (n = 6). Statistical analysis was performed by two-way ANOVA, followed by Tukey’s HSD post-hoc test (*p < 0.05, ***p < 0.001).
200 mg/kg of rosmarinic leaf extract induced weight reduction associated with an increase in fecal lipid excretion. Moreover, they reported that hepatic triglyceride levels were decreased by 39% in mice treated with rosemary leaf extract. Additionally, Kwon et al. also reported that the body weight of mice was significantly lower in a high-fat diet group with addition of 5% Dioscorea nipponica Makino (DN) powder than in the control group (only a high-fat diet). They reported that one of the reasons for the body-weight reduction was that the fecal fat extraction was significantly higher in the DN powder intake group than in the control group. Moreover, they suggested that the saponin contained in DN powder would be involved in this phenomenon. In our results, we presumed that sweet basil powder intake did not influence lipid absorption. However, we did not measure the fecal fat extract in this study. Therefore, in the future, we plan to measure the fecal lipid content of mice fed a sweet basil powder. We speculate that addition of sweet basil to the diet is likely to affect lipid metabolism in the liver. It is known that TGs accumulate in the livers of mice fed a high-fat and high-sucrose diet, which leads to fatty livers. Unfortunately, we were not able to measure the liver TG content because there was an insufficient amount of liver for analysis. Therefore, we could not determine if sweet basil intake inhibited development of a fatty liver. However, we assumed that the sweet basil powder intake inhibited accumulation of TGs in the liver through the effect of the Srebp gene (Fig. 2A). SREBP is a group of transcriptional factors that control cholesterol and fatty acid metabolism-related genes. SREBP-1 is related to lipid metabolism, and SREBP-2 affects cholesterol synthesis in the cells. Especially, it is known that inhibition of activity and expression of SREBP-1c leads to improvement of lipid metabolism, and then it helps prevent metabolic syndrome. Therefore, addition of a food ingredient that can inhibit SREBP-1c expression should have a noticeable effect. Hashidume et al. reported that mRNA expression of the Srebp gene in mice livers fed soy protein isolate was significantly lower than that of mice livers fed casein protein. These findings suggested that addition of sweet basil to the high-fat and high-sucrose diet of the mice in this study inhibited fatty acid synthesis directly or indirectly. However, we did not try to determine which ingredient of sweet basil was responsible for the effect, but we plan to investigate this topic in the future. Furthermore, in the case of ingestion of a high-fat and high-sucrose diet, the hepatic dysfunction should be considered. However, we were not able to determine the plasma ALT and AST because there was insufficient amount of plasma for analysis. Thereat, we measured the expressions of Il1b gene and Ccl2 gene in mice liver. Consequently, there were no significant differences in the expression of Il1b gene among three groups (Fig. 2C). However, the expression of Ccl2 gene in the H group was significantly higher than that in the C group (Fig. 2D). MCP-1 (alias: Ccl2) is an inflammatory chemokine and has the chemotactic activity of monocytes. Therefore, this result suggest that mice liver fed a high-fat and high sucrose diet begin to receive the dysfunction. However, there was no significant differences in the expression of Ccl2 gene between H group and HB group. In the future, we consider to perform the experiment about the effect of sweet basil powder intake on liver dysfunction in mice fed a high-fat and high-sucrose diet.

With respect to sweet basil, Gray et al. reported high amounts of nevadensin and salvigenin in sweet basil when they investigated sweet basil-leaf flavones by chromatographic and spectroscopic methods. On the other hand, Park et al. analyzed 15 kinds of phenolic compounds in seven herbs, including sweet basil, by HPLC. They identified rosmarinic acid as a major antioxidant compound in all seven herbs. Shiga et al. also reported that production of rosmarinic acid increased when they irradiated sweet basil with white light, and the antioxidant activity of sweet basil also increased. Accordingly, it is presumed that rosmarinic acid in sweet basil may have various functions in the body. Rosmarinic acid is an ester of caffeic acid and (3,4-dihydroxyphenyl) acetic acid and has biological and pharmacological activities. Regarding the anti-inflammatory effect of rosmarinic acid, Kim et al. reported that rosmarinic acid significantly reduced the expressions of MCP-1 and macrophage inflammatory protein-1α induced by lipopolysaccharide (LPS) in bone-marrow-derived dendritic cells and inhibited LPS-induced activation of MAPK and nuclear translocation of NF-κB. In our data, the expression of the Ccl (MCP-1) gene was significantly higher in the H group than in the C group. Additionally, the expression of the Ccl gene was drastically lower in the HB group than in the H group (Fig. 3B). Furthermore, we previously reported that sweet basil extract significantly reduced Ccl mRNA expression induced by a co-culture of 3T3-L1 adipocytes and RAW264.7 macrophages. Taken together, we concluded that an ingredient, such as rosmarinic acid, in sweet basil decreased mRNA expression of MCP-1 in the perirenal fat of the mice in this study.

5 Conclusion

We found that sweet basil powder intake suppressed body-weight gain induced by intake of a high-fat and high-sucrose diet for 12 weeks. We speculated that an ingredient in sweet basil improved lipid metabolism, especially the effect of SREBP1-c, and then reduced liver enlargement. We also suggest that sweet basil powder intake inhibited inflammation in the perirenal fat of mice by inhibiting mRNA expression of MCP-1 in the perirenal fat of mice.
Contributions
C. K. accomplished conceptualization, draft of experiment, resources, writing-review and editing. H. T. performed investigation and data curation mainly. Y. Y. performed formal analysis of data and writing-figures. T. K., and K. S. also performed investigation together. M. N., R. T-Y., and C. T-M. performed validation and methodology. All authors approved the manuscript.

References
1) Ishikawa-Tanaka, K.; Ohta, T.; Moritaki, K.; Gotou, T.; Inoue, S. Obesity, weight change and risks for hypertension, diabetes and hypercholesterolemia in Japanese men. *Eur. J. Clin. Nutr.* 56, 601-607 (2002).
2) Aoyagi, K.; Kusano, Y.; Takamura, N.; Abe, Y.; Osaki, M.; Une, H. Obesity and cardiovascular risk factors among men and women aged 40 years and older in rural area of Japan. *J. Physiol. Anthropol.* 25, 371-375 (2006).
3) Bastard, J.P.; Maachi, M.; Lagathu, C.; Kim, M.J.; Caron, M.; Vidal, H.; Capeau, J.; Feve, F. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur. Cytokine Netw.* 17, 4-12 (2006).
4) Jonathan, M.H.; Levings, M.K. Immune regulation in obesity-associated adipose inflammation. *J. Immunol.* 191, 527-532 (2013).
5) Yu, R.; Kim, C.S.; Kwon, B.S.; Kawada, T. Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. *Obesity* 14, 1353-1362 (2006).
6) Jayasinghe, C.; Gotoh, N.; Aoki, T.; Wada, S. Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* 51, 4442-4449 (2003).
7) Ahmed, A.F.; Attia, F.A.K.; Liu, Z.; Li, C.; Wei, J. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. *Food Sci. Human Wellness* 8, 299-305 (2019).
8) Patil, D.D.; Mhaske, D.K.; Wadhwa, G.C. Antibacterial and antioxidant study of *Ocimum basilicum* Labiatae (sweet basil). *J. Advanc. Pharma. Edu. Res.* 2, 104-112 (2011).
9) Shafique, M.; Khan, S.J.; Khan, N.H. Study of antioxidant and antimicrobial activity of sweet basil (*Ocimum basilicum*) essential oil. *Pharmacologyonline* 1, 105-111 (2011).
10) Takeuchi, H.; Takahashi-Muto, C.; Nagase, M.; Kassai, M.; Tanaka-Yachi, R.; Ryo, C. Anti-inflammatory effects of extracts of sweet basil (*Ocimum basilicum* L.) on a co-culture of 3T3-L1 adipocytes and RAW264.7 macrophages. *J. Oleo Sci.* 69, 487-493 (2020).
11) Harach, T.; Aprikian, O.; Monnard, I.; Membrez, M.; Beolor, J.C.; Raab, T.; Mace, K. Rosemary (*Rosmarinus officinalis* L.) leaf extract limits weight gain and liver steatosis in mice fed a high-fat diet. *Planta Med.* 76, 566-571 (2010).
12) Kwon, C.S.; Sohn, H.Y.; Kim, S.H.; Kim, J.H.; Son, K.H.; Lee, J.S.; Lim, J.K.; Kim, J.S. Anti-obesity effect of Dioscorea nipponica Makino with lipase-inhibitory activity in rodents. *Biosci. Biotechnol. Biochem.* 67, 1451-1456 (2003).
13) Hashizume, T.; Sasaki, T.; Inoue, J.; Sato, R. Consumption of soy protein isolate reduces hepatic SRECP-1c and lipogenic gene expression in wild-type mice, but not in FXR-deficient mice. *Biosci. Biotechnol. Biochem.* 75, 1702-1707 (2011).
14) Grayser, R.J.; Bryan, S.E.; Vetch, N.C.; Goldstone, F.J.; Paton, A.; Wollenweber, E. External flavonoids in sweet basil, *Ocimum basilicum*, and related taxa. *Phytochem.* 43, 1041-1047 (1996).
15) Park, J.B. Identification and quantification of a major anti-oxidant and anti-inflammatory phenolic compound found in basil, lemon thyme, mint, oregano, rosemary, sage, and thyme. *Int. J. Food Sci. Nutr.* 62, 577-584 (2011).
16) Shiga, T.; Shoji, K.; Shimada, H.; Hashida, S.; Goto, F.; Yoshihara, T. Effect of light quality on rosmarinic acid content and antioxidant activity of sweet basil, *Ocimum basilicum* L. *Plant Biotech.* 26, 255-259 (2009).
17) Kim, H.K.; Lee, J.J.; Lee, J.S.; Park, Y.M.; Yoon, T.R. Rosmarinic acid down-regulates the LPS-induced production of monocyte chemoattractant protein-1, (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) via the MAPK pathway in bone-marrow derived dendritic cells. *Mol. Cells* 26, 583-589 (2008).

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