Investigating Anti-Obesity Effects by Oral Administration of Aloe vera Gel Extract (AVGE): Possible Involvement in Activation of Brown Adipose Tissue (BAT)

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Summary The aim of this study is to investigate the mechanism of anti-obesity effects of Aloe vera gel extract (AVGE) containing Aloe sterols. Previously, we reported that oral intake of Aloe vera components has an anti-diabetic and anti-obesity effect. This study was designed to assess the role of brown adipose tissue (BAT) in the anti-obesity effect of AVGE. Six-week-old male mice were divided into three groups; STD (standard diet), HFD (60% high fat diet) and AVGE (60% high fat diet with AVGE treatment). During 11 wk of AVGE administration, body weight was monitored. Tissue samples were obtained to be measured the weight and evaluated the gene expressions. Mice treated with AVGE had suppressed body weight, and liver and fat weight gain. To investigate BAT activation, we measured the expression of mRNA related to BAT thermogenesis. Mice in the AVGE group had higher expression of Ucp1, Adrb3, and Cidea in BAT compared to HFD. Next, to investigate the possibility that AVGE induced hepatic FGF21, which is an important factor for nutrient and energy homeostasis including BAT regulation, in vitro study was conducted. HepG2 cell stimulated by AVGE were highly expressed FGF21. These results suggested that BAT activation partially contributes to mechanism of anti-obesity effect of Aloe sterols in diet-induced obesity (DIO) models. However, further study is needed to determine the predominant mechanism.

Key Words Aloe sterols, DIO, PPAR, UCP, FGF21

There are approximately 937 million obese adults and 396 million overweight adults worldwide, and obesity is a growing global public problem (1, 2). Obesity increases the risk of multiple chronic diseases including type-2 diabetes, cardiovascular disease, hypercholesterolemia, hyperlipidemia, hypertension, asthma, and cancer (3, 4). Visceral obesity is the primary factor of a pro-inflammatory state associated with metabolic syndrome. Insulin resistance, endothelial dysfunction, alteration of adipokines, and a pro-atherogenic state are also caused by visceral obesity. In addition, visceral obesity increases cardiovascular and metabolic risk in concert with the usual cardiovascular risk factors such as hypertension, dyslipidaemia, and smoking. Therefore, understanding the molecular mechanisms of obesity may lead to the development of potential therapeutic strategies (5).

In humans, brown adipocytes are distinguished from white adipocytes by metabolic characteristic. Brown adipocytes dissipate energy into heat, while white adipocytes accumulate extra energy into triglyceride (TG). Human brown adipocytes are similar to brown adipose tissue (BAT) in rodents in terms of uncoupling protein 1 (UCP1) expression, multilocular morphology (6, 7). The thermogenic properties of BAT are regulated by UCP1, which uncouples the proton gradient produced by oxidative phosphorylation from adenosine triphosphate (ATP) synthase, and energy is diverted away from ATP synthesis and dissipated as heat (8). BAT maintains core body temperature as part of an adaptive thermogenesis response in small mammals and human infants. Further, BAT contributes to reduce body weight and enhance energy expenditure (9, 10).

 Peroxisome proliferator activated receptors (PPARs; PPARα, PPARγ, and PPARδ) induce transcription of genes that encode for proteins involved in lipid, amino acid, and carbohydrate metabolism (11). PPARα is mainly expressed in tissues with a high fatty acid catabolic rate such as the liver, brown adipose tissue, the heart, and the kidney (12, 13). PPARα is a transcriptional regulator of genes involved in peroxisomal and mitochondrial β-oxidation and fatty acid transport (14). Furthermore, PPARα is a key mediator of fibroblast growth factor 21 (FGF21) in the liver (15). FGF21 is a member of fibroblast growth factors (FGFs) which are involved in nutrient and energy homeostasis. Administration of FGF21 to rodent models of obesity results in

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MATERIALS AND METHODS

Aloe vera gel extract (AVGE) preparation. The method of AVGE preparation was previously described (30). Briefly, the skins of Aloe vera were removed, and the mesophyll parts were collected. The mesophyll parts were dried to obtain Aloe vera gel dry powder. The Aloe vera gel extract (AVGE), containing hydrophobic Aloe sterols, was prepared by supercritical fluid extraction at 34 MPa and 60°C (under supercritical conditions) using CO₂ as a solvent. AVGE was suspended in propylene glycol (PG; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). AVGE is difficult to mix in chow because of high viscosity, we decided to administrate orally using sonde. The concentration of AVGE was adjusted to 0.5 mg/mL with distilled water, and the final PG concentration was adjusted to 1%. The dose of AVGE have determined based on previous study (28, 29).

Animal experiments. Male C57BL/6 mice (6 wk old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The mice were housed 3–4 per cage with a controlled temperature at 20–22°C and maintained on a 12 h light/dark cycle. They were fed a standard diet (D12450B, Research Diets, New Brunswick, NJ, USA) for 1 wk to stabilize their metabolic conditions. After the adaptation period, mice were assigned to one of three groups: standard diet (STD) group, high fat diet (HFD) group, or AVGE group. Mice in the HFD group and AVGE group were fed a high fat diet (HFD) in which 60% of the calories were obtained from lard (D12450B, Research Diets). The STD and HFD group were administered a vehicle (a solution of 1% PG in water). During the treatment, body weight gain and food intake were monitored. After 10 wk of administration, mice were sacrificed. Blood was obtained by cardiac puncture, and serum was prepared by centrifugation of blood at 1,000 × g for 10 min.
Liver, interscapular brown adipose tissue, and mesenteric white adipose tissue and inguinal white adipose tissue were excised and weighed. Liver and brown adipose tissue were frozen immediately with liquid nitrogen. All animal experiments were approved by the Animal Research Committee of the Morinaga Milk Industry (approved numbers: 16-014 and 17-048, experimental period: Aug. 2016–Apr. 2018).

Measurement of oxygen consumption. Oxygen consumption was measured using a O2/CO2 metabolism measuring system for small animals MK-5000RQ (Mromachi Kikai Co., Ltd, Tokyo, Japan). Mice were adapted to individual cages for 2 h before the experiment. The data were collected every 5 min from each cage and measured for 20 h.

Serum parameter. Serum glucose, nonesterified fatty acid (NEFA), and triglyceride (TG) levels were determined with a kit purchased from FUJIFILM Wako Pure Chemical Corporation. Serum FGF21 was measured using an ELISA kit (BioVendor, Brno, Czech Republic).

HepG2 cell cultivation. The HepG2 cell line was purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and maintained in Dulbecco’s modified eagle medium (DMEM) with 10% fetal bovine serum (MP Bio Japan, Tokyo, Japan) and 1% penicillin-streptomycin. Cells were plated at a density of $1.0 \times 10^6$ in 6-well cell culture plates with 1.5 mL DMEM and incubated at 37°C in a humidified atmosphere of 5% CO2. After 24 h, medium was changed and an AVGE suspension with DMSO (Sigma Aldrich, Tokyo, Japan) was added. The concentration of AVGE were determined by the results of preliminary study that Aloe sterols activated PPARalpha-dependent gene transcription. Bezafibrate (Sigma-Aldrich, MO, USA) was used as a positive control agonist for PPARalpha. The final concentration of DMSO was maintained at 1% in all wells. After a 4 h stimulation, cells were collected with TRIzol (Thermo Fisher Scientific, Yokohama, Japan).

RNA preparation and quantification of gene expression. Total RNA from animal tissue and HepG2 cells was isolated using TRizol reagent according to the manufacturer’s protocol. The RNA concentration was determined spectrophotometrically, and cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, US). cDNA was amplified by real-time PCR with an Applied Biosystems 7500/7500 Fast Real-Time PCR System using SYBR Green fluorescence signals. Normalization for loading was accomplished by assessing 36b4/rplp0 for animal tissue and β-actin for HepG2. The primer sets for target
genes are shown in Table 1.

Statistical analysis. All data are presented as mean ± SE. One way ANOVA and Student’s t-test was applied to determine the significance. \( p \leq 0.05 \), was considered statistically significant. All analyses were performed using SPSS for Windows, version 25 (SPSS, Chicago, IL, USA).

RESULTS

Oral intake of AVGE prevented weight gain and mildly suppressed fat accumulation

During experiment period, the body weight of mice fed with high fat diet showed significantly higher than mice fed with standard diet. The body weight of mice in the AVGE group has transitioned lower than that of in the HFD group. At the end of experiment, the body weight of STD group, HFD group and AVGE group were 28.7 ± 1.2 g, 41.0 ± 0.5 g and 38.6 ± 0.9 g, respectively (Fig. 1A). Body weight change of STD group, HFD group and AVGE group were 6.6 ± 3.1 g, 19.0 ± 1.5 g and 16.7 ± 2.2 g. Mice administrated AVGE showed significantly lesser weight gain compared to the HFD group (Fig. 1B). Oral AVGE administration did not affect the food consumption and the calorie intakes compared to the HFD group (Fig. 2A, B). The adverse effects (e.g. decreasing of food consumption and body weight, or abnormal behavior) by oral administration were not observed in each groups. In Fig. 3, the weight of white adipose tissue and liver were shown. Mesenteric WAT weight of STD group, HFD group and AVGE group were 392.7 ± 32.9 mg, 1.195.5 ± 93.3 mg and 939.3 ± 97.2 mg, respectively. Inguinal WAT weight of STD group, HFD group and AVGE group were 830.4 ± 84.5 mg, 2.369.6 ± 126.1 mg and 2.196.3 ± 124.8 mg, respectively. Liver weight of STD group, HFD group and AVGE group were 1.470.3 ± 65.2 mg, 1.675.9 ± 126.1 mg and 1.434.7 ± 107.9 mg, respectively. Mesenteric WAT and inguinal WAT in mice were significantly higher than those in mice fed with standard diet. Comparing

\[ p = 0.078 \]

\[ p = 0.069 \]

\[ p = 0.05 \]

\[ p = 0.05 \]
HFD group and AVGE group, the weight of mesenteric WAT tended to be suppressed by AVGE administration \( (p=0.078) \). There were no significant difference in rectal temperature (Fig. 4) and serum glucose, NEFA, TG (Table 2) among the three groups. Oxygen consumption was significant lower in the HFD group and AVGE group compared to the STD group (Table 3).

**Gene expression of brown adipose tissue**

The mRNA levels of Ucp1, Adrb3, Cidea, Prdm16, Pgc1a, Cpt1β, Fgfr1 and Klb in the total RNA isolated from BAT of the mice were measured at 17 wk old. Ucp1 mRNA levels of the AVGE group were significantly higher than those in mice of the STD group and the HFD group (Fig. 5A). The mRNA levels of Adrb3 and Cidea in AVGE group were significantly higher than other two groups (Fig. 5B, C). Pgc1a mRNA levels in mice in the AVGE group were significantly higher than STD group. There was tendency of increase (not significant) in Pgc1a mRNA levels in mice of the AVGE group compared to HFD group (Fig. 5E). There were no significant differences in the mRNA levels of Prdm16, Cpt1β and Fgfr1 (Fig. 5D, F and G). The Klb mRNA levels in mice of the HFD group and AVGE group were significantly lower than those of STD group (Fig. 5H).

**AVGE potensially induce hepatic FGF21**

We measured Fgf21 mRNA expression levels in the liver and serum FGF21 levels of the mice at 17 wk old. There were no significant differences in mRNA levels and serum concentration of FGF21 among the groups (Fig. 6A & 6B). However, in vitro study with HepG2 cell line indicated that AVGE induced hepatic FGF21. mRNA levels of HepG2 stimulated with AVGE (10 μg/mL) were significantly higher than control (Fig. 7).

**DISCUSSION**

Brown adipose tissue (BAT) efficiently utilizes glucose and lipids and whole body metabolism. Peripheral tissue insulin resistance causes metabolic disorders and negatively correlates with BAT function (31, 32). Thus, enhancing BAT activation is an effective strategy for improving metabolic disorders and preventing obesity (33). BAT is activated by cold exposure. Cold exposure causes the release of norepinephrine via the sympathetic nervous system (SNS) and induces BAT thermogenesis through β3-adrenergic receptor (ADRB3) activation (34, 35). In addition to cold exposure, the utilization of dietary components is expected. Some dietary molecules, including phytochemicals (e.g., capsaicin, resveratrol, curcumin, and green tea catechin) and dietary fatty acids (e.g., DHA and EPA), are reported to promote BAT activation (36–39).

In the previous study, we reported that Aloe sterols has anti-obesity effect in the DIO animals by acting as a ligand of peroxisome proliferator-activated receptors (PPAR) α and γ, which are regulating gene expression related to lipid transport, lipogenesis, gluconeogenesis (29). In addition, we also confirmed that oral administration of Aloe vera gel powder containing Aloe sterols increased oxygen consumption, in DIO rats (40). Since PPARs are known to regulate the BAT activation and energy consumption is increased in activated BAT, we hypothesized that BAT activation partially contributes to mechanism of anti-obesity effect of Aloe sterols. However, the contribution of BAT activity hasn’t been examined in anti-obesity effect of Aloe sterols. In this study, we evaluated the ability of AVGE to prevent obesity and activate BAT in DIO animals. We found that oral administration of AVGE significantly suppressed body weight in DIO model. Body weight was significantly lower in the AVGE group compared to the HFD group throughout the experiment period (Fig. 1A). Furthermore, change in body weight was significantly suppressed in the AVGE group than those in the HFD group (Fig. 1B), although the food consumption was not changed (Fig. 2A, B). The weight of white adipose tissue also indicated the anti-obesity effect of AVGE. The weight of mesenteric WAT and inguinal WAT remarkably increased in the HFD group and the AVGE group than those of the STD group.

### Table 2. Serum parameters of mice at 17 wk old.

|                | STD         | HFD         | AVGE        |
|----------------|-------------|-------------|-------------|
| Glucose (mg/dL)| 251.3±15.0  | 280.0±14.8  | 279.7±12.4  |
| NEFA (mEq/L)   | 0.72±0.12   | 0.93±0.06   | 0.98±0.13   |
| TG (mg/dL)     | 173.3±13.9  | 191.4±9.8   | 205.4±49.8  |

Data are presented as means±SE, \( n=8 \).

### Table 3. Oxygen consumption at 15 wk old.

|        | STD          | HFD          | AVGE         |
|--------|--------------|--------------|--------------|
| Light  | 4,227.5±96.0 | 3,613.6±64.1*| 3,620.3±99.4*|
| Dark   | 4,684.5±119.8| 3,931.4±59.6*| 3,954.4±117.9*|
| Total  | 4,486.3±102.1| 3,795.4±57.2*| 3,811.9±105.5*|

Data are presented as means±SE, \( n=8 \). *Significantly different from the STD group at \( p<0.05 \).
Fig. 5. Relative *Ucp1*, *Adrb3*, *Prdm16*, *Cidea*, *Fgfr1* or *Klb* mRNA levels in mice brown adipose tissue. Tissue samples were obtained after 10 wk of diet-induced obesity and used to determine mRNA expression levels by real time RT-PCR. Values are expressed as means±SE, n=8. * Significantly different at p<0.05.
However, mesenteric WAT of the AVGE group tended to be lower than that of the HFD group. According to this study, we supported that AVGE suppressed body weight gain and prevented obesity without changing of food intake.

To investigate the effects of AVGE treatment on BAT activation, we examined the changes of rectal temperature, oxygen consumption, and gene expression levels. Interestingly, Ucp1, Adrb3 and Cidea, which are related to BAT activation, were significantly up-regulated by AVGE administration (Fig. 5A–C), but the rectal temperature (Fig. 4) and the oxygen consumption (Table 3) weren’t affected. Our previous studies have reported that Aloe sterols regulates the gene expression related to metabolic pathway in liver and increases oxygen consumption in DIO model animals (29, 40). In this study, we couldn’t confirm the increase in the mRNA expression levels in the liver and serum levels of FGF21 by administration of AVGE. However, AVGE upregulated FGF21 expression in vitro study with HepG2 cell line. Induction of gene expression was as strong as bezafibrate which is known as PPARα agonist (Fig. 7). From these data, we showed that Aloe sterols on BAT activation with phenotype change, like thermogenesis and oxygen consumption, further studies would be needed, for example, cold exposure experiment etc.

In conclusion, we found that oral intake of AVGE induced upregulation of mRNA expression levels of genes related to BAT activation, and anti-obesity effects. These findings suggest that BAT activity may be partially involved in the anti-obesity mechanism of oral intake of AVGE. However, further studies are needed in order to elucidate the main mechanism of the anti-obesity effect of AVGE.

**Authorship**

AT, EM, and MT conceived and designed the experiments. AT, EM, and MS performed the experiments. AT, EM, and KN analyzed the data. KY and FA discussed the study and reviewed the manuscript. TG and TK reviewed the analysis and interpretation of the data. AT wrote the manuscript. All authors read and approved the final manuscript.

![Fig. 6. FGF21 levels in liver and serum of mice at 17 wk old.](image)

![Fig. 7. The effect of AVGE administration on FGF21 in a HepG2 cell line.](image)
Disclosure of state of COI

No potential conflict of interest was reported by the authors.

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