

Effect of oleogel (beeswax and canola oil mixture) as a fat replacer in diet on anti-obesogenic and anti-atherosclerotic effect in the rat model

*Issara, U.

Division of Food Science and Technology Management, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani 12110, Thailand

Article history:

Received: 11 August 2024

Received in revised form: 16 September 2024

Accepted: 18 January 2025

Available Online: 12 December 2025

Keywords:

Oleogel,
Anti-obesity,
Lipogenesis,
Lipid metabolism,
Metabolic health,
Oxidative stress

DOI:

[https://doi.org/10.26656/fr.2017.9\(6\).180](https://doi.org/10.26656/fr.2017.9(6).180)

Abstract

The long-term consumption of edible fat may influence obesogenic effects and atherosclerosis. This work was to examine the effect of oleogel (OG) made from a beeswax and canola oil mixture supplement on anti-obesity and blood vessel health in rats. Male Sprague-Dawley (n=24; 4 weeks old) were divided into three groups and fed either a regular or high-fat diet (HFD) or an OG diet for 8 weeks. Bodyweight gain, dietary intake, body compositions (visceral fat and liver), blood characteristics (glucose, TC, TG, HDL, LDL and VLDL), hepatic enzymes (AST, ALP and ALT) activity, liver histopathology, lipid droplets size, and hepatic steatosis area were determined. Also, lipogenesis and angiogenesis-related gene expression were quantified. Supplementation with OG resulted in a significant reduction of body weight and visceral fat ($p<0.05$). Moreover, serum lipid profiles and liver enzymes were improved ($p<0.05$) compared to HFD supplementation. Lipid droplet size and liver steatosis area were decreased by OG treatment ($p<0.05$). The mRNA level of FASN and SREBP1c was downregulated in the visceral fat. OG supplementation showed up-regulation of VEGF and eNOS while suppressing the inflammatory response effect-related gene in animals, CD36, when compared with the HFD group ($p<0.05$). It can suggest that OG supplementation in the diet is delivering health benefits in anti-obesity and improving vascular function, which can be developed into a functional food material.

1. Introduction

Consuming baked products high in trans fats can lead to obesity and atherosclerosis. In particular, the development of an obesogenic and arteriosclerotic state (Mendes-Junior *et al.*, 2018). Several studies have demonstrated that the consumption of high-fat food can cause an increase the weight gain and obesity (Sears and Ghosh, 2016; Limpimwong *et al.*, 2017).

Metabolic disease develops from the metabolic disorder pathway as well as the abnormal lipid metabolism and/or carbohydrate metabolism pathway (Cooke *et al.*, 2016). Currently, the mortality rate in the world caused by ischemic heart disease, cardiovascular disease (CVD), and strokes is continually increasing due to the excessive consumption of trans-fatty acids (Julibert *et al.*, 2019). Generally, this type of fat is found in bakery products, or it is contained in the main ingredients, such as shortening and margarine, which are used to produce baked goods. *Trans-fat* is generated by the hydrogenation process, which involves changing the structure of the lipids from liquid forms to solid forms

(Restrepo and Rieger, 2016). A high amount of saturated fats and *trans-fats* may cause many health diseases, such as obesity, cardiovascular disease, and coronary heart disease. (Fukushima and Lopaschuk, 2016; Giacomozzi *et al.*, 2018). Because *trans-fat* promotes a disadvantage with health functionality, OG has been emphasised in the food science area as well as the food industry due to its high levels of poly-USFA. Many studies have indicated that oleogels could substitute butter or shortening. Oleogelation uses gelators to organize liquid oils into thermally reversible, three-dimensional network-structured solid-like fats with viscoelastic qualities, meeting the demand for flexibility and spreadability in culinary applications. Several kinds of food products, such as instant fried noodles (Lim *et al.*, 2017), muffins (Oh and Lee, 2018), cakes, chocolate pastes, spreads, and margarine-like products, are examples of innovative food products obtained by OG utilization. Even though OG has been approved as a safe food additive material, there are no reports about the impact of OG that is prepared using beeswax and a canola oil mixture as a substitute for high-fat foods from a health perspective.

*Corresponding author.

Email: utthapon_i@rmutt.ac.th

Therefore, this study aimed to determine the effect of a partial substitution of a shortening (HFD) by OG that was made using beeswax and a canola oil mixture on the anti-metabolic syndrome diseases via an *in-vivo* model.

2. Materials and methods

2.1 Animal, diet, and experimental design

Male (24) Sprague Dawley rats were used in this study. The rats were 4 weeks of age and weighed between 100 and 110 g, and they were purchased from DBL Co., Ltd. (Eumseong-gun, Chungcheongbuk-do, Republic of Korea). The animal experimental procedure followed the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (Approved No. SJ-20150701E2), Sejong University, Seoul, Korea. Before the experiment was performed, the animals were left and handled at room temperature for 24 h. The study included three diet groups: a regular diet (control group), a high-fat diet (HFD) with 24.50% shortening to induce obesity, and an oleogel diet (OG) containing 12.25% shortening, 11.05% canola oil, and 1.20% beeswax for 8 weeks, which were produced and obtained from the RaonBio company (Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea). About 30 g from each diet was fed to the rats, and water was provided *ad libitum* during the length of the experiment. The rats' body weights and dietary intake were measured daily.

The author defined the OG as the components created by combining edible beeswax and canola oil in the animal diet.

2.2 Blood sample and tissue collection

Upon completion of the experimental period, the animals were anaesthetised with CO₂, and blood was obtained via heart puncture into a sterile tube devoid of anticoagulant. The samples were then centrifuged at 1000×g to isolate the serum for subsequent assays. The aorta part of the heart was sampled, and it was immediately placed in liquid nitrogen and kept at -80°C to perform the required investigations. The visceral fat was sampled and weighed. The liver tissue was excised, weighed, and it was kept in 10% formalin for 24 h. before transportation to a company for histopathological analysis.

2.3 Serum lipid profiles, glucose level, and hepatic enzyme activity assay

The glucose levels of the animals were assessed on the first day, and at 1 week, 4 weeks, and 8 weeks of the experiment, utilising a digital glucometer (CareSens® Blood Glucose Test Strip, Seocho-gu, Seoul, Republic of Korea).

The blood serum including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein cholesterol (LDL), and very-low-density lipoprotein (VLDL) as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were analyzed by the GC Pharma LAB company (Green Cross Corporation, Yongin-si, Gyeonggi-do, Republic of Korea). All the blood analysis and the liver enzymes were expressed as mg/dL and U/L, respectively.

2.4 Histopathological analysis, lipid droplet size and hepatic steatosis area determination

The liver cross-section was stained with H and E reagent from Korea CFC (Yongin-si, Republic of Korea) and subsequently mounted on a glass slide for future analysis. A digital microscope with a camera (Motic® Model: BA210 Microscope, US) was used to observe the morphology and the lipid accumulation in the liver. The ImageJ program under the ICY software package (Version 1.9.4.1 was used to quantify the lipid droplet size and the hepatic steatosis area distribution. Then, the data was recorded and expressed as an average with standard deviation (Mean ± SD).

2.5 RT-PCR determination

The aorta and the liver tissues were ground with a mortar and subjected to the mRNA isolation process. The MagListo™ 5M Tissue Total RNA Extraction Kit (Bioneer Corporation, Seoul, Korea) was used for the RNA isolation following the manufacturer's guidelines. Afterwards, the cDNA was synthesized from the total RNA obtained using AccuPower® CycleScript RT PreMix (Bioneer Corporation, Seoul, Korea) following the company protocols. To synthesize the cDNA from the samples, the GeneAmp® PCR System 9700 (Applied Biosystems, Marsiling, Singapore) was used for a reverse transcription process was performed and set at 45°C for 60 min to synthesis the cDNA. It was then set at 95°C for 5 min. For the heat inactivation step. The synthesized cDNA sample was used for a comparative cycle threshold experiment for the relative gene expression levels. The quantitative real-time PCR was performed using an AccuPower® 2X Greenstar qPCR Master Mix (Bioneer Corporation, Seoul, Korea) with a StepOnePlus Real-Time PCR system (Applied Biosystems, Marsiling, Singapore). The primer sequences used for the quantitative real-time PCR are FASN, F: 5'-GGAGCCATGGATTGCACATT-3', R: 5'-AGGAAGGCTTCCAGAGAGGA-3'; SREBP1c, F: 5'-TGCAACCGTGTCCAGCCTG-3', R: 5'-TGGATGATGTTGATGATAGAC-3'; CD36, F: 5'-AGGAAGTGGCAAAGAATAGCAG-3', R: 5'-ACAGACAGTGAAGGCTCAAAGA-3'; eNOS, F: 5'-

CGAGATATCTTCAGTCCCAAGC-3', R: 5'-GTGGATTTGCTGCTCTCTAGG-3'; and VEGF, F: 5'-ATCATGCGGATCAAACCTCACC-3', R: 5'-GGTCTGCATTACATCTGCTATGC-3'. The GAPDH (F: 5'-ACCACAGTCCATGCCATCAC-3', R: 5'-TCCACCACCCTGTTGCTGTA-3') was used as a housekeeping gene. The qPCR cycling conditions are as follows. The initial state was set at 95°C for 5 min, the denaturation step was set at 95°C for 5 s, the annealing step was set in the range of 47-55°C for 30 s, the extension step was set at 72°C for 45 s, and the 35 cycles were adjusted. All the data were analyzed using StepOne Software v2.3 with the $\Delta\Delta CT$ method, and the quantitative gene expression was reported.

2.6 Statistical analysis

All data were collected for three replications, and they are presented as mean \pm standard deviation (SD). The SPSS software version 16.0 (SPSS Inc., USA) was used to identify the significance level of the experiment. The *t*-test analysis was applied to compare the body weight between the HFD and the OG group treatment at $p < 0.01$ and $p < 0.05$. For other parameters, a one-way ANOVA and Duncan's multiple ranges were applied. The significant difference level was considered at $p < 0.05$.

3. Results and discussion

3.1 Effect of oleogel supplementation on body weight, body composition and dietary intake of animals

As shown in Figure 1 and Table 1, a significant difference was observed in the bodyweight of the animals between the treatment group and the control after 4 weeks of the experiment ($p < 0.05$) (Figure 1). In the comparison between the HFD and the OG groups, differences in the body weights were found in week 7 of the trial period ($p < 0.01$). The weight increase could possibly be due to the increase in visceral fat, which can be seen in Table 1. The decrease in animal body weight may be ascribed to the consumption of unsaturated fatty acids (USFAs) abundant in canola oil, leading to a reduction in body fat composition. The HFD treatment had the highest amount of visceral fat composition (22.45 \pm 3.40 g) when compared to the OG (20.71 \pm 2.33 g) and the control (12.05 \pm 1.02 g) groups, but there were no significant differences observed in the liver weight ($p > 0.05$). In general, the amount of total fat mass in the mammals, which does not include water, directly affects the body weight increase in conjunction with the BMI index value (Rosenquist *et al.*, 2013). A previous study by Swithers *et al.* (2011) reported that the body weight and the body composition, which include the liver, kidneys, and the heart of the rodent, have increased

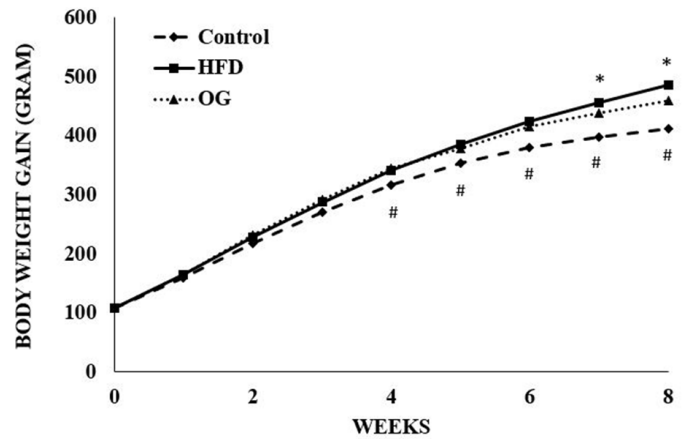


Figure 1. Effect of OG supplementation on the growth performance of animals. HFD: high-fat diet, OG: oleogel.

* shows a significant difference level at $p < 0.01$ between HFD and OG groups treatment.

shows a significant difference level at $p < 0.05$ compared with a control group.

Table 1. Dietary intake and body composition weight.

Treatments	Dietary intake (g/day)	Visceral fat (g)	Liver tissue (g)
Control	23.91 \pm 0.90 ^a	12.05 \pm 1.02 ^c	12.36 \pm 2.16 ^a
HFD	22.13 \pm 1.69 ^b	22.45 \pm 3.40 ^a	14.02 \pm 2.31 ^a
OG	23.18 \pm 1.63 ^a	20.71 \pm 2.33 ^b	13.69 \pm 1.34 ^a

Values are presented as mean \pm SD (n=8). Values with different superscripts are statistically significantly different at $p < 0.05$. HFD: high-fat diet, OG: oleogel. Values are presented as mean \pm SD (n=8). Values with different superscripts are statistically significantly different at $p < 0.05$. HFD: high-fat diet, OG: oleogel.

with long-term HFD feeding. Milagro *et al.* (2006) also found that the animals fed HFD showed a higher body weight, a higher total liver weight, and a higher fat deposition when compared with the regular diet feeding. In the current study, the results were extremely consistent with previous data about HFD treatments. This type of interpretation of growth performance alteration may lead to possible obesogenic effects. Furthermore, scientific evidence has noted that the high accumulation of adipose tissues in mammals results in the lipid metabolism pathway disorder, which leads to obesity and related diseases (Ailhaud *et al.*, 2008). Moreover, it may also cause an insulin resistance state, which is the hormone that controls the blood sugar level, and contribute to obesity-related hyperleptinemia through the release that controls leptin hormone from adipocytes. However, after the OG diet was administered in this study, it revealed that it could significantly reduce the body weight and the visceral fat but not the liver weight of rats ($p < 0.05$), which could lead to anti-obesogenic effects. These effects may be possible due to the level of unsaturated fatty acids (USFA) that is contained in the beeswax and the canola oil OG mixture that activated or re-programmed the adipose tissue cell

function and their production, as well as the cell signaling pathway in the animals. Similarly, good evidence and an explanation of this occurrence have elucidated the results from the case studies of previous research (Rombaldova *et al.*, 2017; Alves *et al.*, 2017; Gonzalez-Hurtado *et al.*, 2018; de-Moura *et al.*, 2018).

3.2 Effect of oleogels supplementation on blood serum characteristics and hepatic enzyme activity of animals

This study demonstrates that a chronic HFD substituted with the OG intake induces a change in the blood biochemical profiles in rats. According to Table 2, a comparison between the HFD and the OG group treatment, OG group has shown a desirable value in the lipid serum profiles of animals with decreased TC and TG, but not the LDL. In contrast, the HDL value increased ($p < 0.05$). Interindividual variability in blood LDL cholesterol after saturated fatty acid replacement has been extensively studied (Koutsos *et al.*, 2024). The LDL cholesterol-lowering effect of substituting saturated fatty acids with unsaturated fatty acids depends on a reciprocal relationship between endogenous cholesterol synthesis, primarily in the liver, and intestinal absorption, which regulates LDL receptor transcription. The observed elevation in serum biomarkers of intestinal cholesterol absorption after the lower-SFA/higher-UFA diet may be a compensatory reaction to decreased cholesterol synthesis (due to diet-induced upregulation of LDL receptor activity), but lathosterol and desmosterol did not decrease. The change of lipid composition in cellular membranes due to dietary fat may affect blood LDL cholesterol, although this was not addressed in this

context (Koutsos *et al.*, 2024). Triglyceride is an important parameter which can be synthesized in the liver and the fat cells of mammals, and a high amount of TG accumulation stimulates lipogenesis and adipogenesis activity (Madsen *et al.*, 2005). Moreover, it has also affected coronary artery disease via stimulating the pro-inflammatory effects of the blood vessels (Yang *et al.*, 2018). According to Limpimwong *et al.* (2017), who studied the effect of an OG prepared with a mixture of rice bran wax and rice bran oil, noted that the OG diet significantly reduced the TC and the TG in rats compared to an HFD treatment. Another study revealed a similar trend of results with the animal's blood characteristics, which were reported by Mendes-Junior *et al.* (2018). The blood glucose testing ranged from 114.00 ± 5.39 mg/dL to 188.00 ± 20.14 mg/dL after the rats were fed the diet for 4 weeks. All treatments have significantly lowered blood glucose ($p < 0.05$). Therefore, it is possible to note that the OG diet might interfere with the glucose metabolism as well as the insulin sensitivity pathway in the rats. Munkong *et al.* (2016) and Cui *et al.* (2015) found a similar tendency with the current study in blood biochemical parameters changes in rodents induced obesity and diabetes model. Nevertheless, the next study needs to confirm these effects through the determination of leptin and adiponectin signaling pathways with anthropomorphic indices of obesity due to associating the alteration of blood lipids and insulin resistance state (Ayina *et al.*, 2016; Boyapati *et al.*, 2018).

To improve the understanding of the influence of the

Table 2. Blood characteristics of animals.

Serum lipid profiles (mg/dL)	Control	HFD	OG
TC	46.00±8.69 ^b	75.40±13.18 ^a	73.80±5.85 ^a
HDL	36.60±6.27 ^c	57.60±5.61 ^b	63.40±8.76 ^a
LDL	7.00±1.58 ^b	11.80±3.27 ^a	13.20±4.55 ^a
TG	64.80±5.43 ^c	112.60±10.42 ^a	76.60±12.46 ^b
VLDL	18.40±8.62 ^a	12.80±2.17 ^b	15.20±2.68 ^{ab}
Hepatic enzymes (U/L)			
ALP	118.00±33.23 ^c	178.20±49.22 ^a	137.40±37.73 ^b
ALT	23.00±2.00 ^b	33.00±6.56 ^a	27.40±5.18 ^{ab}
AST	87.00±18.76 ^b	144.80±32.45 ^a	118.00±21.23 ^{ab}
Glucose level (mg/dL)			
Initial day	114.20±11.03 ^{aC}	116.80±6.53 ^{aB}	114.00±5.39 ^{aB}
Week 1	159.00±24.30 ^{aB}	163.40±13.89 ^{aAB}	161.00±22.89 ^{aA}
Week 4	171.00±10.02 ^{bA}	188.00±20.14 ^{aA}	183.00±13.08 ^{aA}
Week 8	159.40±8.44 ^{bB}	178.80±13.20 ^{aA}	159.00±7.58 ^{bAB}

Values are presented as mean±SD (n=8). Values with different lowercase superscripts in the same row are statistically significantly different at $p < 0.05$. Values with different uppercase superscripts in the same column are statistically significantly different at $p < 0.05$. TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein cholesterol, VLDL: very low-density lipoprotein, ALT: Alanine aminotransferase, AST: aspartate aminotransferase and ALP: alkaline phosphatase, HFD: high-fat diet, OG: oleogel.

animals' liver activity after the supplementation of an OG diet, the liver efficiency through the liver function enzymatic activity was also examined. The normal level of hepatic enzyme activity, which typically indicates liver dysfunction and the liver inflammation state, is not over 0-35 U/L, 0-48 U/L, and 30-120 U/L of AST, ALT, and ALP, respectively (Nyblom *et al.*, 2006). Table 2 illustrates that the OG group treatment had a lower value of AST (118.00 ± 21.23 U/L), ALT (27.40 ± 5.18 U/L), and ALP (137.40 ± 37.73 U/L) when compared with the HFD group (AST: 144.80 ± 32.45 U/L, ALT: 33.00 ± 6.56 U/L, and ALP: 178.20 ± 49.22 U/L) ($p < 0.05$). The long-term HFD intake promoted a dramatic effect on liver function. Contreras and Hernández (2016) reported that increased specific plasma enzymes are considered liver damage and liver disease development, which is associated with the hepatic mitochondrial enzymes release, which leads to a high level of ROS, which is reactive oxygen species, the production that causes the liver inflammatory effect in mammals. The study of the effects of omega-3 fatty acids in nonalcoholic fatty liver disease (NAFLD) via the meta-analysis data has found a positive correlation with the reduction of hepatic enzymes that include ALT, AST, and GGT (gamma-glutamyltransferase) (Lu *et al.*, 2016). Thus, an OG diet in recent trials that was composed of canola oil, which is an abundant source of omega-3 fatty acids, might play an important role in hepatic health functionality and regulate the hepatic enzymes with the advantage of this fatty acid and resulting in anti-fatty liver disease. Based on the current findings, it can be implied that a high rise in the liver enzyme levels can cause poor biological activity in the liver, which contributes to the consideration of this parameter as a biomarker that indicates obesogenic causes.

3.3 Effect of oleogel supplementation on liver morphology, lipid droplet size, and hepatic steatosis area distribution

According to the previous studies, the excessive lipid synthesis via an endogenous and exogenous pathway and its accumulation in the liver of animals might be chronic HFD-induced obesity that causes inflammation and steatosis (Tovar *et al.*, 2011; Batista *et al.*, 2018). Figure 2A-2C shows the effect of an OG supplemented diet on rats' liver morphology, lipid droplet size, and lipid accumulation. After an OG treatment for 8 weeks, the lipid droplet size had significantly reduced (126.70 ± 10.60 μm) when compared to the HFD group (159.07 ± 15.44 μm) ($p < 0.05$). Also, OG provided a decrease in the hepatic steatosis area in the rats (HFD: 59% and OG: 31%). The results are observed in Figure 2A, which displays the liver morphological characteristics. The high accumulation of lipids in the

visceral fat contributes to the increase in body weight, as shown in Figure 1. Although liver weight did not significantly differ among groups, the reduction in hepatic lipid droplet size in the OG group suggests a reduction in fat accumulation within the liver tissue itself, independent of total liver mass. Likewise, Milagro *et al.* (2006) illustrated that an increased animal weight induced by HFD feeding causes liver oxidative stress. This effect contributes to low liver activity, and it may suppress the lipid oxidation process that leads to an inflammatory condition of the liver and liver damage. Poor up-regulating lipid synthesis in conjunction with white adipose tissue numbers assembly in rats' liver, which is illustrated in Figure 2C, could be indicated by the poor lipid metabolism pathway via the inflammatory effect of the liver. The results also suggest potential liver dysfunction, as indicated by elevated liver enzyme levels (Table 2), potentially associated with impaired hepatic function and liver inflammation. Consequently, this leads to the development of risk factors that cause obesity and/or related metabolic diseases. Moreover, previous studies by Kim and Jang (2014) illustrated that the accumulation of hepatic triglycerides is associated with altered expression of key lipogenic genes, including PPAR γ , FASN, LPL, and CPT-1, which are regulated by the overexpression of SREBPs. Ailhaud *et al.* (2008) noted that the suitable equilibrium ratios of the fatty acid composition in the OG diet, which contained canola oil, may suppress the adipogenesis processes that result in the lower lipid hoarding capacity. However, to clarify these mechanisms and the related pathways, the determination of the specific biomarkers that play a vital role in controlling the transcriptional or mediator cytokine response associated with the function of

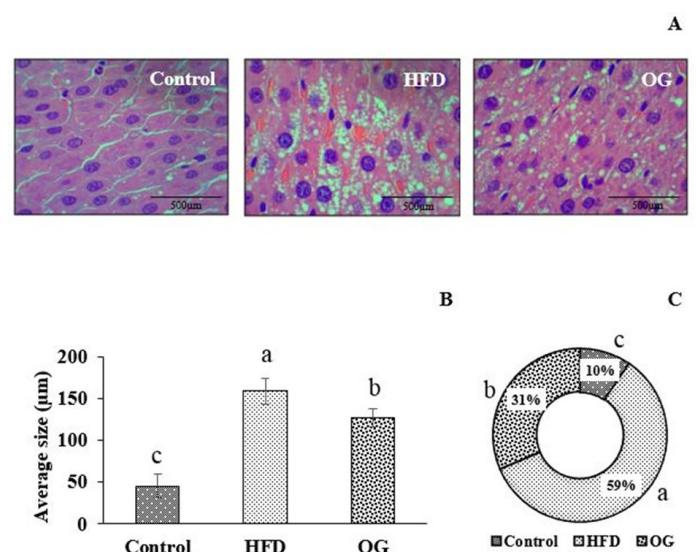


Figure 2. Effect of OG supplementation on liver cross-section morphology (A), lipid droplets size (B) and hepatic steatosis area (C). Values with different lowercase superscripts in the same row are statistically significantly different at $p < 0.05$. HFD: high-fat diet, OG: oleogel.

adipogenesis should be investigated to confirm the efficacy of an OG diet supplementation on mammalian health functionality.

3.4 Effect of oleogel supplementation on the mRNA regulation of lipogenesis and angiogenesis-related gene expression

The oxidative stress of adipocytes in conjunction with endothelial cells leads to the generation of lipid peroxidation in the lipid metabolism pathway and the increase of the risk factors involved with vascular flexibility (Ardestani *et al.*, 2008). The effect of OG substitution into the HFD on the regulation of lipogenesis and vasculogenesis is shown in Figure 3. The fatty acid synthase (FASN) and the sterol regulatory element-binding protein 1c (SREBP1c) showed a significant increase in the mRNA expression in the HFD-induced obesity group ($p < 0.05$). Also, it showed downregulation of these lipogenesis-related genes after the OG treatment ($p < 0.05$) (Figure 3A and 3B, respectively). Comparatively, the HFD-fed rat treatment showed a rise in the CD36 gene (Figure 3E). The main protein functioned in lipid uptake in mammalian cells when it was compared with the OG and the control treatment ($p < 0.05$). For the vaso-protective effects, the OG exerted the mRNA level of the endothelial nitric oxide synthase (eNOS) and the vascular endothelial growth factor (VEGF) compared to the HFD group (Figure 3C and 3D, respectively). In general, during the differentiation process, the white adipose tissue was mainly controlled by the function of the peroxisome proliferator-activated receptor-gamma gene (PPAR γ) via the stimulation of the SREBP1c and the CEBP α

(CCAAT/enhancer-binding protein alpha) gene, which contributed to the adipocyte-specific gene expression. Park and Sung (2015) reported that lipid synthesis and its accumulation in the fat cells were inhibited by the supplementation of carnosic acid through the attenuation of the fatty acid desaturation during the differentiation process. Sekiya *et al.* (2008) found that the oxidative stress in the HepG2 cells was caused by the lipid accumulation that passes the activation of the SREBP1c gene, which leads to the poor control of genes that are associated with fatty acid production. In particular, the FASN. However, this study showed a reduction in the mRNA expression of both the lipogenesis-related genes, which included the SREBP1c and the FASN (Figure 3A and 3B), after the OG treatment in the animals. Based on these results, it can be suggested that controlling the SREBP1c may influence lipid homeostasis by suppressing the FASN mRNA expression level in rats fed an OG diet, which contributes to inhibiting the fatty liver disease-induced hepatic lipogenesis pathway (Eissing *et al.*, 2013).

The vascular disorders caused by oxidative stress or dysfunction of the vascular cells affect angiogenesis in mammals, which contributes to the development of atherosclerotic disease (Munkong *et al.*, 2016; Yang *et al.*, 2018). The suppression of VEGF (Vascular Endothelial Growth Factor) in vascular smooth muscle cells with the excessive uptake of lipid induces a vascular dysfunction. According to Figure 3E, an HFD-fed rat promoted a higher mRNA expression of CD36, which resulted in the downregulation of VEGF and the eNOS synthesis. The CD36 is involved in multiple processes of lipid metabolism pathways in the whole animal system, which also plays an important role in producing lipoprotein and the lipolysis processes (Zhao *et al.*, 2018). Systemic lupus erythematosus, dialysis, obesity, and type 2 diabetes are examples of patients with chronic inflammation, which have significantly increased the risk of atherosclerosis (Zhao *et al.*, 2018). The report from Yang *et al.* (2018) noted that cardiac repair has been recovered by the signalling mechanisms that mediate the endothelial cells and increase the VEGF mRNA expression and the VEGF serum level of the animal regarded with time and in a concentration-dependent manner. Munkong *et al.* (2016) demonstrated that rats fed with rice bran water extract in conjunction with HFD provided lower levels of the CD36 and the NF- κ B p65 but raised the eNOS mRNA expression in the rodents' aorta. Moreover, the reduction of the serum MDA levels was observed in the rice bran water extracted (RBWE) treatment compared with the HFD. This study did not determine the MDA level, which is a biological marker for vascular oxidative stress conditions. These recent trials are consistent with the

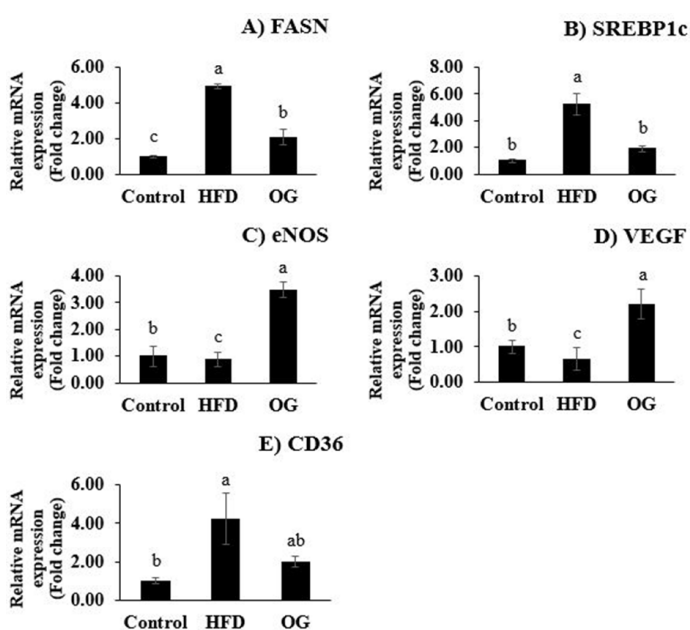


Figure 3. Relative expression levels of genes related to lipogenesis (A and B) in the visceral fat and angiogenesis (C, D and E) in aortic tissue. Bars with different notations are statistically significantly different ($p < 0.05$).

previous studies, which illustrated the effects of low-fat food and dietary fatty acids, especially among unsaturated fatty acids, such as omega-3, 6, and 9. Also, the intake helps to regulate the serum nitric oxide synthase enzyme and the inflammatory mediator cytokines (TNF α , NF- κ B and MCP-1) expression (Weintraub, 2002; Ardestani *et al.*, 2008; Zhao *et al.*, 2009; Zhuang *et al.*, 2013; Chait and Eckel, 2016). Hence, the OG diet in this experiment, which is rich in omega-3, could prevent the development of vascular disorders that contribute to cardiovascular disease through the reduction of lipid uptake in endothelial cells and the up-regulation of the angiogenesis-related gene signaling. However, further studies need to confirm other pathways that are associated with the vascular inflammatory effects. The lipid metabolism, the glucose pathways, the insulin sensitivity, and the release of the calcium ion are obtained from the intracellular and the extracellular, which influence the molecular mechanism of the vascular calcification. Also, it may result in a pathobiological process that correlates with increased morbidity and mortality in patients with chronic systemic metabolic diseases (Chang *et al.*, 2015).

4. Conclusion

The study's results suggest that partially substituting beeswax canola oil-OG in high-fat diets with shortening yields favourable health benefits by decreasing body weight, glucose, total cholesterol, and triglyceride levels, which are primary contributors to obesity in animals. Furthermore, it has enhanced the hepatic enzymes that influence hepatic inflammation, resulting in anti-hepatic steatosis. While the OG treatment showed modulation of key angiogenic markers such as VEGF and eNOS, further studies are needed to confirm whether OG directly promotes angiogenesis or improves vascular function through indirect mechanisms. Therefore, the OG obtained from the beeswax and the canola oil mixture can be used as an alternative fat ingredient as a therapeutic food to reduce the risk factors of metabolic diseases. However, further studies need to consider more in terms of the related pathways of lipid and glucose metabolisms at a molecular level to extensively confirm this beeswax canola oil-OG 's effects on health functions.

Conflict of interest

The authors declare no potential conflict of interest.

Acknowledgments

This research was supported by Korea Institute of Planning and evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High

Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA), Korea (115003033HD020) and by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (2019R1F1A1059043). Also, the author would like to thank the Department of Food Science and Biotechnology, College of Life Science, Sejong University, Seoul, South Korea for any experimental facilities.

References

- Ailhaud, G., Guesnet, P. and Cunnane, S.C. (2008). An emerging risk factor for obesity: does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development. *British Journal of Nutrition*, 100(3), 461-470. <https://doi.org/10.1017/S0007114508911569>.
- Alves, M.G., Moreira, Â., Guimarães, M., Nora, M., Sousa, M., Oliveira, P.F. and Monteiro, M.P. (2017). Body mass index is associated with region-dependent metabolic reprogramming of adipose tissue. *BBA Clinical*, 8, 1-6. <https://doi.org/10.1016/j.bbacli.2017.05.001>
- Ardestani, A., Yazdanparast, R. and Jamshidi, S. (2008). Therapeutic effects of teucrium polium extract on oxidative stress in pancreas of streptozotocin-induced diabetic rats. *Journal of Medicinal Food*, 11(3), 525-532. <https://doi.org/10.1089/jmf.2006.0230>
- Ayina, C.N.A., Noubiap, J.J.N., Etoundi Ngoa, L.S., Boudou, P., Gautier, J.F., Mengnjo, M.K., Mbanya, J.C. and Sobngwi, E. (2016). Association of serum leptin and adiponectin with anthropomorphic indices of obesity, blood lipids and insulin resistance in a Sub-Saharan African population. *Lipids in Health and Disease*, 15(1), 96. <https://doi.org/10.1186/s12944-016-0264-x>
- Batista, Â.G., da Silva-Maia, J.K., Mendonça, M.C.P., Soares, E.S., Lima, G.C., Bogusz Junior, S., da Cruz-Höfling, M.A. and Maróstica Júnior, M.R. (2018). Jaboticaba berry peel intake increases short chain fatty acids production and prevent hepatic steatosis in mice fed high-fat diet. *Journal of Functional Foods*, 48, 266-274. <https://doi.org/10.1016/j.jff.2018.07.020>
- Boyapati, R., Chintalapani, S., Ramiseti, A., Salavathi, S.S. and Ramachandran, R. (2018). Evaluation of serum leptin and adiponectin in obese individuals with chronic periodontitis. *Contemporary Clinical Dentistry*, 9(Suppl 2), S210-S214. https://doi.org/10.4103/ccd.ccd_1_18
- Chait, A. and Eckel, R.H. (2016). Lipids, lipoproteins, and cardiovascular disease: clinical pharmacology now and in the future. *The Journal of Clinical*

- Endocrinology and Metabolism*, 101(3), 804-814. <https://doi.org/10.1210/jc.2015-3940>
- Chang, H.J., Li, T.F., Guo, J.L., Lan, Y.L., Kong, Y.Q., Meng, X., Ma, X.J., Lu, X.L., Lu, W.Y. and Zheng, S.J. (2015). Effects of high glucose on expression of OPG and RANKL in rat aortic vascular smooth muscle cells. *Asian Pacific Journal of Tropical Medicine*, 8(3), 209-213. [https://doi.org/10.1016/S1995-7645\(14\)60317-5](https://doi.org/10.1016/S1995-7645(14)60317-5)
- Contreras-Zentella, M.L. and Hernández-Muñoz, R. (2016). Is liver enzyme release really associated with cell necrosis induced by oxidant stress. *Oxidative Medicine and Cellular Longevity*, 2016, 3529149. <https://doi.org/10.1155/2016/3529149>
- Cooke, A.A., Connaughton, R.M., Lyons, C.L., McMorrow, A.M. and Roche, H.M. (2016). Fatty acids and chronic low grade inflammation associated with obesity and the metabolic syndrome. *European Journal of Pharmacology*, 785, 207-214. <https://doi.org/10.1016/j.ejphar.2016.04.021>
- Cui, M., Kim, H.Y., Lee, K.H., Jeong, J.K., Hwang, J.H., Yeo, K.Y., Ryu, B.H., Choi, J.H. and Park, K.Y. (2015). Antiobesity effects of kimchi in diet-induced obese mice. *Journal of Ethnic Foods*, 2(3), 137-144. <https://doi.org/10.1016/j.jef.2015.08.001>
- de-Moura, D.M., Pais S.N., Lopes da-Conceição, L., Aparecida-dos R.S., Xavier, V.F., Maciel dos-Santos D.M., de-Oliveira Barbosa, R.C., Oliveira de-Paula, S., da Matta, S.L.P., Licursi de-Oliveira, L., Bressan, J. and Gouveia Peluzio, M.do.C. (2018). Consumption of virgin coconut oil in Wistar rats increases saturated fatty acids in the liver and adipose tissue, as well as adipose tissue inflammation. *Journal of Functional Foods*, 48, 472-480. <https://doi.org/10.1016/j.jff.2018.07.036>
- Eissing, L., Scherer, T., Tödter, K., Knippschild, U., Greve, J.W., Buurman, W.A., Pinnschmidt, H.O., Rensen, S.S., Wolf, A.M., Bartelt, A., Heeren, J., Buettner, C. and Scheja, L. (2013). De novo lipogenesis in human fat and liver is linked to ChREBP- β and metabolic health. *Nature Communications*, 4, 1528. <https://doi.org/10.1038/ncomms2537>
- Fukushima, A. and Lopaschuk, G.D. (2016). Cardiac fatty acid oxidation in heart failure associated with obesity and diabetes. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1861(10), 1525-1534. <https://doi.org/10.1016/j.bbailip.2016.03.020>
- Giacomozzi, A.S., Carrín, M.E. and Palla, C.A. (2018). Muffins elaborated with optimized monoglycerides oleogels: from solid fat replacer obtention to product quality evaluation. *Journal of Food Science*, 83(6), 1505-1515. <https://doi.org/10.1111/1750-3841.14174>
- Gonzalez, H.E., Lee, J., Choi, J. and Wolfgang, M.J. (2018). Fatty acid oxidation is required for active and quiescent brown adipose tissue maintenance and thermogenic programming. *Molecular Metabolism*, 7, 45-56. <https://doi.org/10.1016/j.molmet.2017.11.004>
- Julibert, A., Bibiloni, M.D.M. and Tur, J.A. (2019). Dietary fat intake and metabolic syndrome in adults: A systematic review. *Nutrition, Metabolism and Cardiovascular Diseases*, 29(9), 887-905. <https://doi.org/10.1016/j.numecd.2019.05.055>
- Kim, K.B. and Jang, S.H. (2014). Anti-obesity effect of EGCG and glucosamine-6-phosphate through decreased expression of genes related to adipogenesis and cell cycle arrest in 3T3-L1 adipocytes. *Journal of Nutrition and Health*, 47(1), 1-11. <https://doi.org/10.4163/jnh.2014.47.1.1>
- Koutsos, A., Griffin, B.A., Antoni, R., Ozen, E., Sellem, L., Wong, G., Ayyad, H., Fielding, B.A., Robertson, M.D., Swann, J., Jackson, K.G. and Lovegrove, J.A. (2024). Variation of LDL cholesterol in response to the replacement of saturated with unsaturated fatty acids: a nonrandomized, sequential dietary intervention; the Reading, Imperial, Surrey, Saturated fat Cholesterol Intervention ("RISSCI"-1) study. *The American Journal of Clinical Nutrition*, 120(4), 854-863. <https://doi.org/10.1016/j.ajcnut.2024.07.032>
- Lim, J., Jeong, S., Oh, I.K. and Lee, S. (2017). Evaluation of soybean oil-carnauba wax oleogels as an alternative to high saturated fat frying media for instant fried noodles. *LWT*, 84, 788-794. <https://doi.org/10.1016/j.lwt.2017.06.054>
- Limpimwong, W., Kumrungsee, T., Kato, N., Yanaka, N. and Thongngam, M. (2017). Rice bran wax oleogel: A potential margarine replacement and its digestibility effect in rats fed a high-fat diet. *Journal of Functional Foods*, 39, 250-256. <https://doi.org/10.1016/j.jff.2017.10.035>
- Lu, W., Li, S., Li, J., Wang, J., Zhang, R., Zhou, Y., Yin, Q., Zheng, Y., Wang, F., Xia, Y., Chen, K., Liu, T., Lu, J., Zhou, Y. and Guo, C. (2016). Effects of omega-3 fatty acid in nonalcoholic fatty liver disease: a meta-analysis. *Gastroenterology Research and Practice*, 2016, 1459790. <https://doi.org/10.1155/2016/1459790>
- Madsen, L., Petersen, R.K. and Kristiansen, K. (2005). Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1740(2), 266-286. <https://doi.org/10.1016/j.bbadis.2005.03.001>
- Mendes, J.L.G., Freitas, L.L.C., Oliveira, J.R., Melo, M.B., Feltenberger, J.D., Brandi, I.V., Carvalho, B.M.A., Guimarães, A.L.S., De-Paula, A.M.B., D'Angelis, C.E.M., Campagnole-Santos, M.J., Souza Santos, R.A., Braga, V.A. and Santos, S.H.S. (2018). The

- usefulness of short-term high-fat/high salt diet as a model of metabolic syndrome in mice. *Life Sciences*, 209, 341-348. <https://doi.org/10.1016/j.lfs.2018.08.034>
- Milagro, F.I., Campión, J. and Martínez, J.A. (2006). Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity*, 14(7), 1118-1123. <https://doi.org/10.1038/oby.2006.128>
- Munkong, N., Hansakul, P., Yoysungnoen, B., Wongnoppavich, A., Sireeratawong, S., Kaendee, N. and Lerdvuthisophon, N. (2016). Vasoprotective effects of rice bran water extract on rats fed with high-fat diet. *Asian Pacific Journal of Tropical Biomedicine*, 6 (9), 778-784. <https://doi.org/10.1016/j.apjtb.2016.07.009>
- Nyblom, H., Björnsson, E., Simrén, M., Aldenborg, F., Almer, S. and Olsson, R. (2006). The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. *Liver International*, 26(7), 840-845. <https://doi.org/10.1111/j.1478-3231.2006.01304.x>
- Oh, I.K. and Lee, S. (2018). Utilization of foam structured hydroxypropyl methylcellulose for oleogels and their application as a solid fat replacer in muffins. *Food Hydrocolloids*, 77, 796-802. <https://doi.org/10.1016/j.foodhyd.2017.11.022>
- Park, M.Y. and Sung, M.K. (2015). Carnosic acid inhibits lipid accumulation in 3t3-l1 adipocytes through attenuation of fatty acid desaturation. *Journal of Cancer Prevention*, 20(1), 41-49. <https://doi.org/10.15430/JCP.2015.20.1.41>
- Restrepo, B.J. and Rieger, M. (2016). Trans fat and cardiovascular disease mortality: Evidence from bans in restaurants in New York. *Journal of Health Economics*, 45, 176-196. <https://doi.org/10.1016/j.jhealeco.2015.09.005>
- Rombaldova, M., Janovska, P., Kopecky, J. and Kuda, O. (2017). Omega-3 fatty acids promote fatty acid utilization and production of pro-resolving lipid mediators in alternatively activated adipose tissue macrophages. *Biochemical and Biophysical Research Communications*, 490(3), 1080-1085. <https://doi.org/10.1016/j.bbrc.2017.06.170>
- Rosenquist, K.J., Pedley, A., Massaro, J.M., Therikelsen, K.E., Murabito, J.M., Hoffmann, U. and Fox, C.S. (2013). Visceral and subcutaneous fat quality and cardiometabolic risk. *JACC: Cardiovascular Imaging*, 6(7), 762-771. <https://doi.org/10.1016/j.jcmg.2012.11.021>
- Sears, C. and Ghosh, S. (2016). Excess omega-6 polyunsaturated fatty acid intake is associated with negative cardiovascular, intestinal and metabolic outcomes in mice. *Canadian Journal of Diabetes*, 40 (4), 278-279. <https://doi.org/10.1016/j.cjcd.2016.06.002>
- Sekiya, M., Hiraishi, A., Touyama, M. and Sakamoto, K. (2008). Oxidative stress induced lipid accumulation via SREBP1c activation in HepG2 cells. *Biochemical and Biophysical Research Communications*, 375(4), 602-607. <https://doi.org/10.1016/j.bbrc.2008.08.068>
- Swithers, S.E., Ogden, S.B. and Davidson, T.L. (2011). Fat substitutes promote weight gain in rats consuming high-fat diets. *Behavioral Neuroscience*, 125(4), 512-518. <https://doi.org/10.1037/a0024404>
- Tovar, A.R., Díaz-Villaseñor, A., Cruz-Salazar, N., Ordáz, G., Granados, O., Palacios-González, B., Tovar-Palacio, C., López, P. and Torres, N. (2011). Dietary type and amount of fat modulate lipid metabolism gene expression in liver and in adipose tissue in high-fat diet-fed rats. *Archives of Medical Research*, 42(6), 540-553. <https://doi.org/10.1016/j.arcmed.2011.10.004>
- Weintraub, W.S. (2002). Is atherosclerotic vascular disease related to a high-fat diet. *Journal of Clinical Epidemiology*, 55(11), 1064-1072. [https://doi.org/10.1016/S0895-4356\(02\)00541-3](https://doi.org/10.1016/S0895-4356(02)00541-3)
- Yang, Z., Wan, J., Pan, W. and Zou, J. (2018). Expression of vascular endothelial growth factor in cardiac repair: Signaling mechanisms mediating vascular protective effects. *International Journal of Biological Macromolecules*, 113, 179-185. <https://doi.org/10.1016/j.ijbiomac.2018.02.111>
- Zhao, C.X., Xu, X., Cui, Y., Wang, P., Wei, X., Yang, S., Edin, M.L., Zeldin, D.C. and Wang, D.W. (2009). Increased endothelial nitric-oxide synthase expression reduces hypertension and hyperinsulinemia in fructose-treated rats. *The Journal of Pharmacology and Experimental Therapeutics*, 328(2), 610-620. <https://doi.org/10.1124/jpet.108.143396>
- Zhao, L., Varghese, Z., Moorhead, J.F., Chen, Y. and Ruan, X.Z. (2018). CD36 and lipid metabolism in the evolution of atherosclerosis. *British Medical Bulletin*, 126(1), 101-112. <https://doi.org/10.1093/bmb/ldy006>
- Zhuang, W., Wang, G., Li, L., Lin, G. and Deng, Z. (2013). Omega-3 polyunsaturated fatty acids reduce vascular endothelial growth factor production and suppress endothelial wound repair. *Journal of Cardiovascular Translational Research*, 6(2), 287-293. <https://doi.org/10.1007/s12265-012-9409-0>