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BITC and S-Carvone Restrain High-fat Diet-induced Obesity and Ameliorate Hepatic Steatosis and Insulin Resistance

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Abstract

Purpose—To investigate the preventative activity of benzyl isothiocyanate and *S*-carvone against high-fat diet-induced obesity and metabolic complications.

Methods—Ten-week-old C57BL/6 male mice were fed a high-fat diet and injected intraperitoneally twice per week with benzyl isothiocyanate, *S*-carvone, or vehicle for 8 weeks. The body weight, food intake, and body composition were monitored, and glucose tolerance and insulin tolerance tests were performed at the end of the experiment. Serum and tissue samples were studied using serum biochemistry, histological, and gene expression analysis to define the effects of benzyl isothiocyanate and *S*-carvone treatments on lipid and glucose metabolism and inflammatory responses.

Results—Benzyl isothiocyanate and *S*-carvone blocked high-fat diet-induced weight gain, fat accumulation in the liver, and insulin resistance. The beneficial effects were found to be associated with an improvement of expression of macrophage marker genes in white adipose tissue, including *F4/80*, *Cd11b*, *Cd11c*, *Cd206*, and *Tnf- α* , and reduced expression of genes (*Ppar γ 2*, *Scd1*, *Cd36*) responsible for lipid synthesis and transport in the liver.

Conclusion—Benzyl isothiocyanate and *S*-carvone block high-fat diet-induced obesity and metabolism disorders and can be considered for management of the obesity epidemic that affects approximately 36% of adults and 17% of children in the USA.

Keywords

Obesity; inflammation; BITC; *S*-carvone; antioxidant

INTRODUCTION

The prevalence of obesity in the United States has significantly increased in the past five years, reaching 36% for adults and 17% for youth (1). Obesity is a risk factor for several common diseases, including cardiovascular diseases, type 2 diabetes, hypertension, hyperlipidemia, nonalcoholic fatty liver disease (NAFLD, or hepatic steatosis), kidney diseases, osteoarthritis, depression, and some types of cancer (2). A 2008 estimate of

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medical costs for obesity in the United States is \$147 billion. The annual productive costs of obesity-related absenteeism range between \$3.38 billion and \$6.38 billion (3). Thus, an indispensable strategy is needed to prevent and control the obesity epidemic and its associated complications.

In obesity pathogenesis, low-grade chronic inflammation plays an important role in adipose tissue remodeling and expansion (4). This role includes macrophage infiltration into white adipose tissue, pro-inflammatory cytokine release, and dysregulation of adipokine release (5). Previous studies have shown that reactive oxygen species (ROS), an indicator of oxidative stress, serve as a trigger for inflammation in adipose tissue (6, 7). We have previously reported that suppression of ROS activity by overexpression of endogenous antioxidant enzyme or by use of antioxidants was able to block high-fat diet (HFD)-induced weight gain and its metabolic complications (8, 9). These earlier studies have provided a strong indication that antioxidants could be an effective agent to prevent obesity and obesity-associated metabolic disorders. In the current study, we selected two different antioxidants, benzyl isothiocyanate (BITC) and *S*-carvone, and explored their activities in blocking HFD-induced obesity in a mouse model.

BITC (Fig. 1a) is a natural product found in cruciferous vegetables such as cabbage, broccoli, turnip, rapeseeds, radish and others in the cabbage family. It is produced by hydrolysis of glucotropaeolin under the influence of myrosinase (10). In addition to its antioxidant activity, BITC is also known for its anti-cancer activity shown via inhibition of P450 mediated-carcinogen activation *in vivo* and induction of cell cycle arrest *in vitro* (10–12). BITC has been shown to inhibit lipopolysaccharide-induced inflammatory response in Raw 264.7 murine macrophage cell line and has suppressed 12-O-tetradecanoylphorbol 13-acetate-induced ear edema formation in mice (13). The antioxidant activity of BITC involves inhibition of NADPH oxidase to reduce superoxide generation and activation of P450 phase II enzymes (11, 14).

S-carvone (Fig. 1a), also called D-carvone or (+)-carvone, belongs to the terpenoids family of natural components of essential oils, such as caraway, spearmint, and dill. Carvone has *S* and *R* isomers, and the *S* form is the main isomer in caraway seed and is used in the food and flavor industry (15). The antioxidant activity of *S*-carvone has been demonstrated in *in vitro* systems (16–18). Studies have shown that *S*-carvone has antibacterial, antifungal, anticancer, immunomodulatory and anti-inflammatory activity (15, 19–21). Mechanistically, *S*-carvone induces glutathione S-transferase (22) which is downregulated by HFD (23).

We demonstrate here that both BITC and *S*-carvone are effective in blocking HFD-induced obesity, insulin resistance and fatty liver via a mechanism that involves inhibition of HFD-induced inflammation and down-regulation of mRNA level of genes responsible for lipogenesis.

MATERIALS AND METHODS

Materials

BITC and *S*-carvone were purchased from Sigma-Aldrich (St. Louis, MO). HFD (60% kJ/fat, 20% kJ/protein, and 20% kJ/carbohydrate) was from Bio-serv (Frenchtown, NJ). The insulin assay kits were obtained from Mercodia AB (Winston-Salem, NC) and glucometer and blood test strips were obtained from NIPRO Diagnostics™ (Fort Lauderdale, FL). The Humulin® insulin was purchased from Eli Lilly (Indianapolis, IN). The Infinity™ kit for triglycerides (TG) was from Fisher Diagnostics (Middletown, VA). The non-esterified fatty acids kits were obtained from Wako Bioproducts (Richmond, VA). The RNeasy® Lipid Tissue Mini Kit was purchased from QIAGEN® (Hilden, Germany). The First-stand cDNA Synthesis kit was obtained from Origene® (Rockville, MD). The PerfeCta® SYBR® Green FastMix® ROX™ was purchased from Quanta Biosciences (Gaithersburg, MD).

Animals and Animal Treatments

C57BL/6 mice (male, ten weeks old) were from Charles River Laboratories (Wilmington, MA). All procedures performed on mice were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Georgia, Athens, Georgia. To assess BITC and *S*-carvone activity in blocking HFD-induced obesity and obesity-associated metabolic disorders, mice were grouped randomly (n=5) and fed a HFD, and received twice weekly injection (*i.p.*) of BITC (12.5 mg/kg), *S*-carvone (175 mg/kg), or carrier solution of dimethyl sulfoxide (DMSO) for 8 weeks. The volume of each injection was fixed at 20 µl per mouse. Mouse body weight was monitored weekly, and the body composition was determined using EchoMRI-100™ from the Echo Medical System (Houston, TX). All mice were euthanized using the method of carbon dioxide inhalation followed by cervical dislocation.

Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)

At the beginning of the 8th week, mice were fasted for six hours and received *i.p.* injection of glucose (1.5 g/kg) dissolved in saline. Approximately 5 µl of blood from each mouse was collected from the tail and glucose level was determined at time 0, 30, 60, and 120 min using a glucometer. ITT was performed on Wednesday of the 8th week. Mice were fasted for four hours and injected *i.p.* with insulin (0.75 U/kg). The blood glucose level was determined applying the same schedule as GTT. Animals were put back on HFD after each procedure.

Histological Examination by Hematoxylin and Eosin (H&E) Staining

Liver, epididymal white adipose tissue (eWAT), perirenal white adipose tissues (pWAT), inguinal white adipose tissues (iWAT), and brown adipose tissue (BAT) were collected from animals and fixed in 10% formalin at the end of the experiment. The fixed tissues were dehydrated, embedded in paraffin, sectioned at 6 µm in thickness, and stained with H&E following the manufacturer's instructions. Tissue sections were examined, and photo images were taken under an optical microscope using the Nikon's NIS-Elements imaging software (Melville, NY).

Determination of Hepatic Triglyceride Level

For lipid extraction, liver samples (100 mg) were homogenized in 1 ml of PBS. Tissue homogenate (300 μ l) was mixed with 5 ml of chloroform/methanol mixture (3:2, v/v ratio) and incubated overnight at 4°C. The mixture was centrifuged at 2,000 rpm for 10 min and supernatant (organic phase) was transferred to a new tube, dried, and dissolved in 2% Triton-X100. Triglyceride concentration was determined using the Infinity™ kit following the manufacturer's instructions.

Determination of Insulin Resistance and Serum Lipid Level

Three days after ITT, mice were fasted for six hours and euthanized by CO₂ inhalation. Whole blood was collected from each animal and serum concentrations of insulin were determined by an ELISA following the procedure provided by the supplier of the insulin analysis kit. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was determined using the formula: [fasting insulin (ng/ml) $\times$ fasting blood glucose (mg/dl)/405] (24). Serum concentrations of triglyceride and free fatty acids were determined using the procedure provided by the supplier of the lipid analysis kits.

Gene Expression Analysis

Total RNAs from mouse adipose tissue and liver were isolated using an RNeasy® Lipid Tissue Mini Kit. The complementary DNA (cDNA) was synthesized using a First-stand cDNA synthesis kit. Real-time quantitative polymerase chain reaction (qPCR) was performed using a SYBR Green as a detector with a StepOnePlus system (Applied Biosystems). Data was analyzed by the C_T method using *Gapdh* mRNA as the internal control. All primer sequences used are listed in Table I.

Statistical Analysis

Statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparisons test using the plotting software (Prism) from GraphPad, Inc. (La Jolla, CA). A value of $P < 0.05$ was considered statistically significant, and each data point represents the mean $\pm$ standard deviation (SD) or standard error of the mean (SEM).

RESULTS

BITC and S-carvone Blocked HFD-induced Weight Gain

C57BL/6 mice were fed a HFD and injected twice weekly with BITC (12.5 mg/kg), S-carvone (175 mg/kg) (*i.p.*), or the vehicle for 8 weeks. Results in Fig. 1b show smaller size of mice with BITC and S-carvone treatment compared to the DMSO-treated control. At the end of week 8, the average body weight of BITC-treated and S-carvone-treated mice were 29.3 ± 3.5 g and 30.9 ± 1.1 g, respectively, compared to control mice at 40.2 ± 3.1 g (Fig. 1c). The recognized difference in body weight of treated mice was due to the difference in fat mass, and no difference was seen in lean mass (Fig. 1d). The average food intake was the same (Fig. 1e) between BITC- and S-carvone-treated and control mice.

The apparent size (Fig. 2a) and average weight (Fig. 2b) of eWAT, pWAT, iWAT and BAT were examined and compared between the treated and control mice. With the exception of

BAT, the WATs of control animals are significantly larger than those of BITC- and *S*-carvone-treated mice. The images of H&E staining show miniature adipocytes and lack of crown-like structure in BITC- and *S*-carvone-treated mice compared to that of DMSO-treated control mice (Fig. 2c). Lower lipid content in BAT compared to control (Fig. 2c, right panel) was also observed. The average diameter of adipocytes in BITC- and *S*-carvone-treated animals was at 61.7 – 74%, 70.6 – 86.7%, 58.1 – 69.2% of the size of control animals in eWAT, pWAT, and iWAT, respectively (Fig. 2d). These results demonstrate that BITC and *S*-carvone treatment suppressed lipid accumulation in adipose tissues.

BITC and *S*-Carvone Treatment Reduces Inflammation in WAT

Diet-induced obesity is associated with inflammation in adipose tissue with characteristics of accumulation of type-1 macrophages and crown-like structure (Fig. 2c). To extend the study of the effects of BITC and *S*-carvone on diet-induced inflammation in adipose tissue at the molecular level, we extracted total RNA from WAT and determined the mRNA levels of macrophage-specific marker genes including *F4/80*, *Cd11b*, *Cd11c*, and proinflammatory cytokine *Tnf- α* . In addition, mRNA levels of the anti-inflammation gene (*Cd206*) and those of the leptin gene were also examined. Results in Figure 3a show decreased mRNA levels of the *F4/80*, *Cd11b* and *Cd11c* genes in eWAT of *S*-carvone-treated mice. An enhanced expression of the anti-inflammatory *Cd206* gene was seen in eWAT of BITC-treated mice compared to that of control. *Tnf- α* and *leptin* gene expression was lower in iWAT of *S*-carvone- and BITC-treated mice, respectively. In addition to lack of crown-like structure in adipose tissue (a landmark of macrophage infiltration) in BITC- and *S*-carvone-treated animals shown in Figure 2c, these results confirm that BITC and *S*-carvone suppress HFD-induced macrophage infiltration and inflammation in WAT.

BITC and *S*-Carvone Prevent HFD-induced Hepatic Steatosis

Hepatic steatosis or fatty liver is commonly associated with obesity (25). To evaluate the impact of BITC and *S*-carvone on hepatic fat accumulation, lipid level in the liver was examined. The images of H&E staining showed a high level of hepatic lipid level in control mice, but not in those treated with BITC and *S*-carvone (Fig. 4a). This was consistent with results of lipid measurements. Results in Figure 4b show that the level of hepatic triglyceride is 21.5 ± 4 mg/g in control mice compared to 15.8 ± 0.7 and 16.2 ± 1.3 mg/g in BITC- and *S*-carvone-treated mice, respectively. Livers of control animals weigh about 1.5 ± 0.3 g compared to 1.1 ± 0.1 g of treated animals (Fig. 4c). There was no significant difference observed in serum levels of triglycerides and free fatty acids (Fig. 4d–e).

To understand the protective mechanism of BITC and *S*-carvone against HFD-induced fatty liver, mRNA levels of genes responsible for hepatic lipid metabolism were examined. Both BITC and *S*-carvone treatment resulted in expression reduction of the peroxisome proliferator-activated receptor- γ 2 gene (*Ppar- γ 2*) coding for *de novo* lipogenesis transcriptional factor, fatty acid transporter gene (*Cd36*), and stearoyl-CoA desaturase 1 gene (*Scd1*) (Fig. 5). Moreover, *S*-carvone downregulated mRNA levels of genes encoding *de novo* lipogenesis transcriptional factor sterol regulatory element-binding protein 1 c (*Srebp1c*) and genes responsible for fatty acids synthesis, including acetyl-CoA carboxylase 1 (*Acc1*) and fatty acid synthase (*Fas*) (Fig. 5). These data suggest that BITC and *S*-carvone

prevented hepatic lipid accumulation through suppression of lipid synthesis and accumulation.

BITC and S-Carvone Restrained Diet-induced Hyperglycemia and Insulin Resistance

Obesity is strongly associated with insulin resistance and hyperglycemia (26). To evaluate the impacts of BITC and S-carvone on glucose homeostasis, non-fasting blood glucose level was determined at the end of the 8-week treatments. The blood glucose concentration in BITC-treated mice showed levels at 167.6 ± 21.1 mg/dL and at 161.8 ± 5.4 mg/dL in S-carvone-treated mice, compared to control mice at 223.8 ± 22.1 mg/dL (Fig. 6a). In addition, BITC- and S-carvone-treated mice were more tolerant to glucose, as revealed by GTT (Fig. 6b) and a plot of the area under the curve (Fig. 6c). Results from ITT showed that BITC- and S-carvone-treated mice had a significantly higher response to insulin injection (Fig. 6d). The area under the curve of ITT confirmed the improvement of insulin sensitivity due to BITC and S-carvone treatment (Fig. 6e). HFD-induced hyperinsulinemia and insulin resistance were blocked by BITC and S-carvone (Fig. 6f–g). These data demonstrate the protective activity of BITC and S-carvone against HFD-induced hyperglycemia, hyperinsulinemia, and insulin resistance.

DISCUSSION

Inflammation is an essential step toward the development of obesity and obesity-associated metabolic diseases. We have previously shown that blockade of HFD-induced inflammation is a successful strategy to repress weight gain and improve obesity-associated complications (27–30). In this study, we examined the protective activity of BITC and S-carvone against HFD-induced obesity, fatty liver, and insulin resistance. Our results demonstrate that twice-weekly intraperitoneal injection of BITC and S-carvone restrains HFD-induced weight gain (Figs. 1–2) and hepatic lipid accumulation (Fig. 4), and improves glucose homeostasis (Fig. 6). These beneficial effects were directly linked to lower expression of macrophage marker genes in adipose tissue (Fig. 3) as well as those responsible for hepatic *de novo* lipogenesis (Fig. 5).

Our conclusion is in agreement with previous studies that showed a potent effect of BITC and S-carvone against inflammatory diseases (11, 13, 21). BITC has been shown to reduce lipopolysaccharide-induced secretion of inflammatory cytokines (e.g., IL-1 β , TNF- α , and IL-6) and their mRNA levels in Raw264.7 macrophage cell line in a dose-dependent manner involving inhibition of NF κ B, Akt, and ERK1/2 signaling pathways (13). In the same study, the authors also demonstrated that BITC inhibits ear edema inflammation and increases iNOS and COX-2 protein levels in mice. Waterman *et al.* (31) recently showed that *Moringa oleifera* extract, which is rich in isothiocyanates (BITC precursor), suppressed body weight gain and food intake in a diet-induced obesity animal model. The conclusion derived from the study serves as additional evidence in support of the activity of BITC in blocking HFD-induced obesity, as shown here in the current study.

S-carvone has been shown *in vitro* for its antioxidant activity in several studies and reduced pro-oxidants induced stress (16, 18). In addition, an *in vivo* study has shown that daily S-carvone treatment for 30 days protected rats from developing hypertension and restored

vitamin C, vitamin E, and glutathione levels (17). Moreover, *S*-carvone had the highest activity in inducing glutathione *S*-transferase compared with other carvone derivatives or isomers (22).

Hepatic steatosis is associated with obesity where excess fat is accumulated in the liver due to induction of *de novo* lipogenesis and enhanced expression of fatty acid transporters in hepatocytes. Feeding mice with a HFD for 2 weeks has been reported to induce fatty liver coinciding with elevation of mRNA levels of hepatic *Ppar γ* and *Cd36* in mice (32). In agreement, our data showed that both BITC and *S*-carvone significantly blocked HFD-induced up-regulation of *Ppar γ* and *Cd36* (Fig. 5) and *Scd-1*, a gene encoding stearoyl-CoA desaturase-1 that controls the rate-limiting step in monounsaturated fatty acid formation. The enzyme activity of stearoyl-CoA desaturase-1 has been shown to be elevated in humans with fatty liver disease (33). *S*-carvone, but not BITC, reduced the expression of the genes involving lipid synthesis, including *Srebp1c*, *Acc1*, and *Fas* (Fig. 5). A previous study using *Moringa oleifera* extract has also demonstrated suppression of lipid accumulation in mouse liver with no change of serum concentrations of triacylglycerol (31), in agreement with our results (Fig. 4).

Inflammation of the adipose tissue is known to cause insulin resistance and glucose intolerance (34, 35). Insulin resistance increases gluconeogenesis and decreases blood glucose absorption, leading to glucose intolerance. Many studies have shown that antioxidants are effective in blocking inflammation, increasing insulin sensitivity and improving glucose homeostasis (9, 30, 31). Consistent with these studies, our results show that BITC and *S*-carvone treatment alleviated insulin resistance and improved glucose intolerance in HFD-fed mice (Fig. 6) by suppressing TNF α gene expression and macrophage activation in adipose tissue (Fig. 3).

In conclusion, the current study demonstrates the preventative activity of BITC and *S*-carvone against HFD-induced obesity and obesity-associated metabolic complications. BITC and *S*-carvone effects are achieved by the alteration in inflammatory and lipogenesis gene expression in adipose and liver tissues, respectively. These results suggest that BITC and *S*-carvone could be considered protecting agents against diet-induced obesity and metabolic disorders.

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ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
AUC	Area under the curve
BAT	Brown adipose tissue
BITC	Benzyl isothiocyanate

DMSO	Dimethyl sulfoxide
eWAT	Epididymal white adipose tissues
FAS	Fatty acid synthase
FFA	Free fatty acid
GTT	Glucose tolerance test
HFD	High-fat diet
HOMA-IR	Homeostatic model assessment of insulin resistance
ITT	Insulin tolerance test
iWAT	Inguinal white adipose tissues
PPARγ	Peroxisome proliferator-activated receptor- γ
pWAT	Perirenal white adipose tissues
SCD	Stearoyl-CoA desaturase
SREBP	Sterol regulatory element-binding protein
TG	Triglyceride
TNF-α	Tumor necrosis factor α

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Figure 1. BITC and *S*-carvone inhibit body weight gain in mice fed an HFD. Ten-week-old C57BL/6 male mice were fed an HFD and injected *i.p.* twice weekly with BITC (12.5 mg/kg), *S*-carvone (175 mg/kg), or vehicle (DMSO) for 8 weeks. **(a)** Chemical structures of BITC (left) and *S*-(+)-carvone (right); **(b)** representative picture of the mice at the end of the experiment; **(c)** growth curve; **(d)** body composition at week 8; and **(e)** average food intake. Values represent mean $\pm$ SEM, * $p < 0.05$ (n=5).

Figure 2. Impacts of BITC and *S*-carvone treatment on adipose tissues of animals. Mice were fed an HFD continuously for 8 weeks, and injected *i.p.* twice weekly with BITC (12.5 mg/kg), *S*-carvone (175 mg/kg), or vehicle DMSO for 8 weeks. Animals were sacrificed at the end of experiment and adipose tissues were collected and examined (n=5). (a) Photo images of pWAT, iWAT, eWAT, and BAT from treated and control animals; (b) weights of eWAT, iWAT, pWAT, and BAT; (c) photo-images of H&E staining of eWAT, iWAT, pWAT and BAT; black arrows indicate crown-like structure (CLS), scale bar: 100 μm; (d) average diameter of adipocytes in different WATs (n= 300). Values are mean ± SD. **p* < 0.05, ***p* < 0.01.

Figure 3. BITC and *S*-carvone suppressed HFD-induced inflammation. At the end of experiments, mice were sacrificed and total RNAs were extracted from eWAT or iWAT and gene expression was determined by real-time quantitative PCR. **(a)** mRNA levels of macrophage marker genes, *Tnf- α* , and *leptin* genes in eWAT; and **(b)** mRNA levels of selected genes in iWAT. All values represent mean $\pm$ SEM, * $p < 0.05$ (n=4).

Figure 4. BITC and *S*-carvone blocked HFD-induced fatty liver. Mice were fed a HFD during the entire study and received twice weekly *i.p.* injections of BITC (12.5 mg/kg), *S*-carvone (175 mg/kg), or DMSO. Mice were sacrificed at the end of experiment and liver samples were collected. (a) Photo images of H&E staining of liver sections photographed at $\times 100$ magnification, scale bars: 100 μ m; (b) triacylglycerol level in the liver (n=5); (c) liver weight (n=5); (d) serum levels of triacylglycerol (n=4); and (e) serum levels of free fatty acids (n=4). Each data point represents mean $\pm$ SEM, * p < 0.05.

Figure 5. Impacts of BITC and *S*-carvone treatment on mRNA levels of genes responsible for hepatic lipid metabolism. Total RNA was isolated from liver tissue, and relative mRNA level of selected genes were determined by qPCR. All values represent mean $\pm$ SEM, * $p < 0.05$ (n=4).

Figure 6. BITC and *S*-carvone improved glucose homeostasis and hyperinsulinemia in HFD-fed mice. (a) Non-fasting blood glucose level (n=5); (b) profile of serum glucose concentration after *i.p.* glucose injection (1.5 g/kg) (n=5); (c) area under the curve of glucose tolerance test; (d) time-dependent percentage of initial glucose concentration in blood after *i.p.* insulin injection (0.75 U/kg) (n=5); (e) area under the curve of insulin tolerance test; (f) fasting serum insulin concentration (n=4); (g) homeostasis model assessment-estimated insulin resistance (HOMA-IR) (n=4). Each data point represents mean $\pm$ SEM, * $p < 0.05$.

Table I

Primers used for gene expression analysis by real time quantitative PCR

Gene	Forward Primers	Reverse Primers
<i>F4/80</i>	5' CCCAGGTGCTTACAGAGTG 3'	5' GTGCCAGAGTGGATGTCT 3'
<i>Cd68</i>	5' CCATCCTTCACGATGACACCT 3'	5' GGCAGGGTTATGAGTGACAGTT 3'
<i>Cd11b</i>	5' ATGGACGCTGATGGCAATACC 3'	5' TCCCCATTACGTCTCCCA 3'
<i>Cd11c</i>	5' CTGGATAGCCTTTCTTCTGCTG 3'	5' GCACACTGTGTCCGAACTCA 3'
<i>Cd206</i>	5' CTCTGTTCACTATTGGACGC 3'	5' CGGAATTCTGGGATTCAGCTTC 3'
<i>Tnf- α</i>	5' CCCTCACACTCAGATCATCTTCT 3'	5' GCTACGACGTGGGCTACAG 3'
<i>Leptin</i>	5' GAGACCCCTGTGTCGGTTC 3'	5' CTGCGTGTGTGAAATGTCATTG 3'
<i>Pparγ2</i>	5' TCGCTGATGCACTGCCTATG 3'	5' GAGAGGTCCACAGAGCTGATT 3'
<i>Cd36</i>	5' CCTTAAAGGAATCCCCGTGT 3'	5' TGCATTTGCCAATGTCTAGC 3'
<i>Srebp1c</i>	5' CCCTGTGTGTAAGGCTTT 3'	5' TTGCGATGTCTCCAGAAGTG 3'
<i>Acc1</i>	5' GCCTCTTCCTGACAAACGAG 3'	5' TGAAGTCCGAAACATCTCTG 3'
<i>Fas</i>	5' GGAGGTGGTG ATAGCCGGTAT 3'	5' TGGGTAATCCATAGAGCCCAG 3'
<i>Scd1</i>	5' TTCTTACACGACCACCACCA 3'	5' CCGAAGAGGCAGGTGTAGAG 3'
<i>Gapdh</i>	5' AGGTCGGTGTGAACGGATTG 3'	5' TGTAGACCATGTAGTTGAGGTCA 3'