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Modulation of Cell-mediated Immunity to Suppress High Fat Diet-induced Obesity and Insulin Resistance

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Abstract

Purpose—To assess the effect of immune modulators on high fat diet-induced obesity and insulin resistance.

Methods—C57BL/6 mice were fed a high fat diet and injected intraperitoneally with cyclosporine A, fingolimod, or vehicle twice weekly for 15 weeks. Body weight and food intake were manually measured every other day. Glucose tolerance test, insulin sensitivity, and body composition were examined and compared between the control and the immune modulator treated animals. Tissue samples were collected at the end of the experiment and examined for serum biochemistry, histology, and mRNA levels of marker genes for inflammation, and glucose and lipid metabolism in white and brown adipose tissues and in the liver.

Results—Cyclosporine A and fingolimod suppressed high fat diet-induced weight gain, reduced hepatic fat accumulation, and improved insulin sensitivity. The beneficial effects are associated with altered expression of *F4/80*, *Cd68*, *Il-6*, *Tnf-α*, and *Mcp-1* genes, which are involved in macrophage-related chronic inflammation in adipose and hepatic tissues.

Conclusion—Immune modulation represents an important intervention for obesity and obesity-associated insulin resistance.

Keywords

Cyclosporine A; FTY720; Obesity; Inflammation; Insulin Resistance

INTRODUCTION

Obesity is an epidemic problem worldwide and a leading public health issue. In the United States of America, the prevalence of obesity in adults and children has dramatically increased within the past 20 years. More than one-third of adults, 34.9% (or 78.6 million), and 17% (or 12.7 million) of children and adolescents between the ages of two and nineteen years were obese. Obesity increases the risk of several types of diseases including

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cardiovascular disease, type-2 diabetes, non-alcoholic fatty liver disease, and various types of cancer.

Several lines of evidence show that obesity can alter metabolic signaling, leading to the activation of innate and adaptive immune responses. Obesity can also cause impairment of immune functions and metabolic homeostasis (1,2). Previous studies have noted that an increasing number of immune cells infiltrating into metabolic tissue is a feature of the inflammatory state of obesity. We have previously shown that blocking macrophage activation inhibits the development of high fat diet-induced obesity (3,4). The objective of the current study is to extend our previous work by focusing on the involvement of T-cells and lymphocytes in high fat diet-induced obesity and insulin resistance. The goal of the study is to search for an immune modulator that will suppress the activity of cell-mediated immune responses resulting in attenuation of diet-induced obesity. The study involves direct assessment of two immune suppressing compounds with known activity in modulating cell-mediated immune responses.

Cyclosporin A (CsA) is an immune modulator clinically used in organ transplantation to prevent immune rejection. It reduces the activity of the immune system by interfering with the activity and growth of T cells (5). Previous reports show that CsA decreased IL-6 synthesis at the post-transcriptional level in human macrophages (6). In addition, previous studies have shown that CsA treatment reduces inflammation by down-regulating production of several inflammatory cytokines (6–8). Furthermore, the study by Jiang *et al.* shows that CsA is able to improve glucose metabolism, resulting in remarkably attenuated food intake and body weight gain in the obese mice (9). As CsA is a traditional immune modulator with anti-inflammatory activity, we hypothesize that CsA might possess anti-inflammatory effects to obesity.

Fingolimod (FTY720) is a novel immune modulator used as the first-line treatment for relapsing forms of multiple sclerosis (10). It can prolong allograft transplant survival in numerous models by inhibiting lymphocyte emigration from lymphoid organs (11). FTY720 is a structural analogue of sphingosine, an important amino alcohol in the synthesis of sphingolipids and the integrity of cell membranes. A previous study demonstrates that FTY720 decreases plasma concentrations of pro-inflammatory cytokines in renal transplant patients, such as IFN- γ , TNF- α , IL-6, and IL-12 (12). In addition, FTY720 normalizes renal expression of *Mcp-1* gene and significantly down regulates the activities of M1 and M2 macrophages in rats, which was beneficial in tissue remodeling and repair (13). Although immune modulation effects of FTY720 have been extensively studied, its effects on diet-induced obesity are not fully explored.

In this study, we investigated the effect of CsA and FTY720 on mice fed a high fat diet (HFD). We demonstrated that CsA and FTY720 suppress HFD-induced body weight gain and attenuate insulin resistance and liver steatosis.

MATERIALS AND METHODS

Materials

Cyclosporine A (CsA) and FTY720 were purchased from Selleckchem (Houston, TX). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, MO). HFD (60% kJ/fat, 26% kJ/carbohydrates, 14% kJ/proteins) was obtained from Bio-serve (Frenchtown, NJ, catalog number F3282). Trizol was acquired from Invitrogen (Grand Island, NY). RNeasy Tissue kit was purchased from QIAGEN (Valencia, CA). The Superscript First-Strand Synthesis System was from Invitrogen (Carlsbad, CA). All primers for PCR were synthesized at Sigma (St. Louis, MO). Insulin (Humulin) was purchased from Eli Lilly (Indianapolis, IN). Oil-red O staining solution was obtained from Electron Microscopy Science (Hatfield, PA). Glucose meters and test strips were purchased from LifeScan (Milpitas, CA). The ELISA assay kits for insulin detection were purchased from Mercodia Developing Diagnostics (Winston-Salem, NC). Commercial kits for determination of serum levels of triglycerides, cholesterol, and non-esterified fatty acids were purchased from Thermo Scientific (Middletown, VA).

Animal and Animal Treatment

C57BL/6 mice (male, 24~26 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed under standard conditions with a 12 h light-dark cycle. Two groups of mice (n=5 for each) were intraperitoneally (*i.p.*) injected twice weekly with CsA or FTY720 solubilized in DMSO (50 μ l) at safe and effective doses of 50 mg/kg, 0.04 mg/kg, respectively, according to previous studies (9,14). Control mice were injected with DMSO vehicle solution. Body weight and food intake were monitored twice weekly. Body composition analysis was performed at the end of 15-week treatment using EchoMRI-100 (Echo Medical Systems, Houston, TX). All procedures performed on mice were approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, Georgia (protocol number, A2011 07-Y2-A3).

Intraperitoneal Glucose Tolerance Test (IPGTT) and Insulin Tolerance Test (ITT)

IPGTT were conducted in mice after fasting for 6 h. Glucose solubilized in phosphate-buffered saline (pH=7.4) was injected (*i.p.*) at 2.0 g/kg. Blood glucose levels were measured using glucose test strips and glucose meters at 0, 30, 60 and 120 min after glucose injection. ITT were carried out in mice after fasting for 4 h. Insulin (0.75 U/kg, *i.p.*) was injected, and blood glucose levels were measured using the same time frame as IPGTT after insulin injection. Blood insulin level was determined using an insulin ELISA kit.

Serum Biochemistry Analysis

At the end of the experiments, mice were euthanized and blood samples were collected from heart cavities, kept on ice for 15 min, and centrifuged at 4,000 g for 10 min to collect serum. Triglycerides, cholesterol, and non-esterified free fatty acid (FFA) concentrations were measured following the instruction from the manufacturer of the test kits.

Gene Expression Analysis

Liver and adipose tissue samples were freshly collected and frozen immediately at -80°C until use. Total mRNA from the mouse liver was isolated using the Trizol reagent from Invitrogen (Carlsbad, CA), while the RNAs of adipose tissues were isolated using specialized kits from QIAGEN (Valencia, CA). Two micrograms of total RNA were used for cDNA synthesis as recommended by the manufacturer. Quantitative real-time PCR was performed using SYBR Green as an indicator on the ABI Step One Plus Real-Time PCR system. Data were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as an internal control and analyzed using the $\Delta\Delta\text{Ct}$ method (15). The primer sequences used are listed in Table 1.

Histochemical Study

For haematoxylin and eosin (H&E) staining, tissue samples were freshly collected from animals and immediately fixed overnight in 10% neutrally buffered formalin. Fixed tissues were dehydrated using gradient ethanol and embedded in paraffin. Tissue sections were made at $6\text{ }\mu\text{m}$ in thickness and dried at 37°C for 2 h. H&E staining was performed according to the instruction of a commercial kit from BBC Biochemical. For Oil-red O staining, liver samples were frozen in liquid nitrogen and sectioned at $9\text{ }\mu\text{m}$ in thickness using a Leica CM1850 cryostat. Tissue samples were placed on slides and washed with 60% isopropanol for 5 min before being stained with Oil-red O for 30 min and counterstained with haematoxylin for 1 min. Tissue sections were examined using a Nikon ECLIPSE-Ti optical microscope. Pictures and size measurements of adipocytes were taken using Nikon NIS-Elements AR software (Melville, NY).

Statistics

Statistical analysis was performed by one-way analysis of variance (ANOVA). All data were presented as mean $\pm$ standard error of the mean (SEM) with statistical significance set at $P < 0.05$.

RESULTS

CsA and FTY720 Attenuate HFD-induced Body Weight Gain

We examined whether selected immune modulators could affect body weight gain of C57BL/6 mice fed an HFD. CsA and FTY720 were given *i.p.* at doses of 50 or 0.040 mg/kg twice weekly for 15 weeks, respectively, according to previous studies (9,14). Body weights of all the treated mice were significantly lower ($P < 0.05$) than that of control mice at the end of the experiment (Fig. 1a). CsA-treated mice increased their body weight at a much lower rate than that of vehicle injected control mice. The average body weight of CsA-treated animals was $\sim 34\%$ less than that of control animals at the end of the experiment. The average body weight of mice treated with FTY720 was $\sim 16\%$ less than the control at the end of the experiment. CsA-treated mice show almost total suppression of body weight gain, while the body weight gain of mice treated with FTY720 slowed down after week 7. There is no difference in body weight of these mice in the first 7 weeks compared to the control. Intraperitoneal injection of modulators did not affect food intake of mice (Fig. 1b).

An analysis of body composition by magnetic resonance proton spectroscopy (EchoMRI) was performed to examine the effect of immune modulators on fat accumulation. Results shown in Fig. 1c reveal that the average fat mass of CsA and FTY720 treated mice is significantly lower ($p < 0.05$) than the control by ~60.8% and ~22.2%, respectively. The treated animals show the same lean mass to that of the control, suggesting that these immune modulators do not affect the lean mass of treated mice. The lowered body weights of treated mice were due to less fat mass.

CsA and FTY720 Attenuate HFD-induced Adipose Inflammation

Previous studies show that adipocyte hypertrophy is associated with adipose tissue inflammation (16); therefore, we performed H&E staining on WAT and BAT. The results in Fig. 2a confirm a lower number of crown-like structures in WAT and a decrease in the density of vacuole type structures in BAT of mice treated with immune modulators (Fig. 2b). We confirmed macrophage and T cell activation in adipose tissue by RT-PCR, using cell specific marker genes. Fig. 2c shows that CsA treatment up regulates mRNA levels in WAT of *Cd68*, *F4/80*, *Mcp-1*, and *Tnf- α* by 31%, 84%, 120%, and 180%, respectively, indicating that CsA does not block macrophage infiltration. Conversely, FTY720 significantly decreases the mRNA levels of *Cd68*, *F4/80*, *Mcp-1*, and *Tnf- α* by 77%, 62%, 60%, and 59%, respectively.

Similar to the result in WAT, FTY720 treatment significantly down regulates the mRNA level in BAT of *F4/80*, *Il-6*, *Tnf- α* , and *Mcp-1* by ~81%, ~70%, ~85%, and ~87%, respectively, compared to those of the vehicle control group. However, no significant differences in mRNA levels of *F4/80*, *Il-6*, and *Tnf- α* are observed between the CsA treated and control mice with the exception of *Mcp-1* gene which is down regulated by ~59% (Fig. 2d).

CsA and FTY720 Block HFD-induced Fat Accumulation in Liver without Altering Serum Lipid Levels

After 15 weeks of HFD-feeding, the weight of liver dramatically increased in vehicle treated control mice due to hepatic fat accumulation. H&E staining of liver samples from mice treated with CsA and FTY720 shows less lipid accumulation (white spots) than the control (Fig. 3a). Similarly, in the Oil-red O staining experiments, less fat (red spots) is observed in the liver tissue from CsA- and FTY720-treated mice (Fig. 3b). Both experiments show that CsA and FTY720 treatment prevents HFD-induced fat accumulation in the liver. Meanwhile, serum concentrations of triglycerides, cholesterol and FFA remain the same with the exception of FTY720-treated mice which show an approximately 20% reduction in serum level of cholesterol (Fig. 3c).

CsA and FTY720 Modulate the Transcription of Genes Involved in Chronic Inflammation and Nutrient Metabolism in Liver

The data above show that CsA and FTY720 treatment causes significant decline in liver lipid accumulation in the C57BL/6 mice, revealing a possible role of CsA and FTY720 against lipogenesis. To address this issue, we measured expression levels of genes involved in liver metabolism, including *Fas*, *Acc-1*, *Scd-1* and *G6p*. ACC-1 and SCD-1 are responsible for

the metabolism of fatty acids. G6P is a key enzyme in regulating glucose metabolism. Quantitative PCR of hepatic RNAs from immune modulator-treated mice show different levels of induction of lipogenic gene expression, compared to HFD-fed vehicle control mice (Fig. 4a). CsA increases mRNA level of *Fas* by ~3.1 fold, *Acc-1* by ~2.1 fold, and *Scd-1* by ~3.1 fold. There is no change in mRNA level of *G6p*. Similarly, FTY720 up regulates the expression levels of *Fas* by ~6.2 fold, *Acc-1* by ~7.8 fold, and *Scd-1* by ~4.6 fold, but not *G6p*.

Since HFD-induced fatty liver is commonly accompanied with chronic inflammation, we measured the mRNA levels of several inflammatory genes, including *F4/80*, *Il-6*, *Tnf- α* , and *Mcp-1*. The results (Fig. 4b) show that the treatment with FTY720 significantly down regulates expression of *Il-6*, *Tnf- α* , and *Mcp-1* gene by ~91%, ~81%, and ~85%, respectively, while *F4/80* expression was slightly up regulated. However, treatment with CsA shows a very different result. *F4/80*, *Il-6* and *Tnf- α* increases ~1.8, ~2.3, or ~2.4 fold, respectively. The expression level of *Mcp-1* is slightly lower, but without statistical significance.

CsA and FTY720 Improve Insulin Sensitivity in HFD-fed Mice

An important consequence of diet-induced obesity is the development of insulin resistance. IPGTT were performed in the 14th week of the experiment to examine the effect of immune modulators on insulin sensitivity. After a 6 h fasting, we intraperitoneally injected glucose (1.5 g/kg) and determined blood glucose levels at 0, 30, 60, and 120 min after injection using commercial glucose test strips and meters. Blood glucose levels under fasting conditions prior to glucose load are similar between CsA and FTY720 treated mice, significantly lower than that of the control mice (Fig. 5a). However, the glucose profile shows improvement of glucose elimination in immune modulator treated mice. AUC calculations make this point more evident, showing that CsA and FTY720 treatment led to a ~26.6% and ~24.6% reduction, respectively, compared to the control mice (Fig. 5b).

The insulin sensitivity test was examined 1 week later (15th week) with an insulin tolerance test (ITT) on the same mice. After a 4 h fasting, we intraperitoneally injected insulin (0.75 U/kg) to each animal and determined blood glucose levels at 0, 30, 60, and 120 min. The glycemic drop following insulin administration was more pronounced for CsA and FTY720 treated mice than DMSO treated control (Fig. 5c), suggesting that HFD caused an apparent insulin resistance in control animals. However, CsA and FTY720 treatments effectively block this resistance by attenuating the rise of blood glucose levels after the insulin injection, exhibiting a better insulin sensitivity (Fig. 5c).

In addition, we measured serum levels of insulin using commercial kits. The serum insulin concentrations of CsA and FTY720 treated mice are significantly decreased compared to those of the vehicle treated control (Fig. 5d). These results confirm that CsA and FTY720 improve insulin sensitivity in mice fed an HFD.

DISCUSSION

HFD-induced obesity is associated with chronic inflammation. In this study, we assessed whether immune modulation could affect HFD-induced obesity and its complications. We demonstrate that CsA and FTY720 attenuate HFD-induced obesity without affecting food intake (Fig. 1), inhibit the development of fatty liver (Fig. 3), and improve HFD-induced insulin resistance (Fig. 5). These protective effects are associated with altered expression of genes involved in chronic inflammation in adipose tissues and liver, such as *F4/80*, *Tnf- α* , *Mcp-1*, *Il-6*, and *CD68* (Figs. 2, 4).

An increasing amount of evidence suggests that macrophages and T cells play an important role in adipose tissue remodeling (17–21) involving an increase of pro-inflammatory cell infiltration (M1 macrophages, Th1 cells, CD8⁺ T cells) (22) and a decrease of anti-inflammatory cells (M2 macrophages, Th2 cells, regulatory T cells) (23). In diet-induced obesity, adipose tissue mass expands and the adipocyte size increases. This expansion alters the structure of adipose tissue, which increases the release of free fatty acids into circulation and decreases oxygen delivery to adipocytes, resulting in hypoxia in adipose tissue. This can lead to activation of immune inflammatory responses, and therefore, the apoptosis of adipocytes. Consequently, activation of these responses results in recruitment of macrophages into adipose tissue, forming the crown-like structure around adipocytes and releasing pro-inflammatory cytokines (24). Since these macrophages are in an M1 activation state, once recruited, they further activate the intracellular pro-inflammatory pathway and amplify the inflammation state. In a feed-forward circle, these activations promote macrophage recruitment and their infiltration into adipose tissue, facilitating the development of obesity.

In addition to macrophages, previous studies have shown that HFD increases Th1/Th2 ratio (25) and decreases the proportion of *Foxp3*⁺ T-reg cells in adipose tissue (17). T-bet, a member of the T-box transcription factor family and described as the transcription factor for Th1 CD4⁺ T cells, is directly responsible for the transactivation of *Ifn- γ* (26). In turn, HFD increases IFN- γ induced polarization of macrophages toward an M1 phenotype (27). T-bet-deficient mice fed an HFD display increased visceral fat accumulation and reduced immune cell infiltration (26). GATA3, a Th2-specific transcriptional factor (28,29), inhibited adipogenesis partly by suppressing peroxisome proliferator-activated receptor gamma (*Ppar- γ*) expression and reducing Th2 related cytokines (30). Results in Fig. 2 show that FTY720 reduces macrophage infiltration, while CsA activates macrophages. Therefore, we speculate that the anti-obesity activity of CsA results from decreasing Th1 infiltration and the Th1/Th2 ratio, and increasing the proportion of T-reg cells through T-bet, GATA3, and Foxp3 mediated signaling pathways. Therefore, CsA and FTY720 affect HFD-induced inflammatory cytokine expression, and consequently reduce fat accumulation in adipose tissue through macrophage and T cell dependent pathways.

In addition to adipose tissue inflammation, obesity has been linked to insulin resistance, partly due to an increased pro-inflammatory cytokines (31,32). In adipose tissue, activated macrophages and decreased T-reg cells promote insulin resistance in obesity (33–35). Previous studies indicate that T-bet can modulate obesity-associated insulin resistance in T-

bet deficient mouse model (36,37). Recent studies demonstrate that the chemokine receptor CXCR3 can modulate diet-induced insulin resistance by controlling T cell accumulation (38). Together, these results suggest that macrophage and T cell signals regulate obesity-related insulin resistance. A study from Winer *et al.* suggests that immunotherapy could normalize obesity-associated insulin resistance induced by HFD (25). Furthermore, increasing evidence indicates that CsA and FTY720 can affect insulin resistance in diet-induced obesity (39,40). Consistent with previous studies, our data demonstrates that CsA and FTY720 prevent HFD-induced hyperglycemia and hyperinsulinemia (Figs. 5b, 5d) and improve insulin sensitivity (Fig. 5c).

Results in Figs. 3, 4 show that CsA and FTY720 reduce hepatic fat accumulation along with altered macrophage activation without significantly changing the levels of serum lipids. Therefore, these immune modulators may act through other factors to reduce hepatic lipid accumulation. HFD increases hepatic inflammation by activating macrophages and increasing pro-inflammatory cytokine secretion (41). Similar to subcutaneous adipose tissue, hepatic fat accumulation is also accompanied by T cell activation (42). In obese patients, visceral adipose tissue infiltration was found to be increased by the up regulated expression of the macrophage-specific marker (*Cd68*), and was accompanied by an increase in C-C chemokines (43). Our results show that FTY720 treatment reduces the expression of macrophage specific *F4/80* and *Mcp-1* genes. Additional study is needed to illustrate the underlying mechanism of inhibitive activity of the immune modulator in HFD-induced fatty liver.

In conclusion, data collected from this study demonstrate that CsA and FTY720 treatment protects C57BL/6 mice from HFD induced obesity, insulin resistance, and hepatic steatosis at different levels. CsA appears more effective than FTY720. The beneficial effects are associated with regulation of inflammatory immune responses and blood glucose adjustment. These results suggest that CsA and FTY720 are promising drugs for prevention of HFD-induced obesity, inflammation and related complications. Strategically, immune modulation can be an attractive approach for preventing high fat diet-induced obesity.

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ABBREVIATIONS

ACC	acetyl-CoA carboxylase
BAT	brown adipose tissue
CsA	cyclosporine A
DMSO	dimethyl sulfoxide
FAS	fatty acid synthase

FFA	free fatty acid
FTY720	fingolimod
G6P	glucose 6 phosphatase
HFD	high fat diet
IFN	interferon
IL-4	interleukin 4
IL-6	interleukin 6
IL-12	interleukin 12
IL-17	interleukin 17
IPGTT	intra-peritoneal glucose tolerance test
ITT	insulin tolerance test
MCP	monocyte chemoattractant protein
SCD	stearoyl-CoA desaturase
TNF	tumor necrosis factor
TGF	transforming growth factor
WAT	white adipose tissue

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Fig. 1.

CsA and FTY720 blocked HFD-induced obesity in C57BL/6 mice. C57BL/6 mice were fed an HFD for 15 weeks with twice weekly injection (*i.p.*) of CsA (50 mg/kg), FTY720 (0.04 mg/kg), or DMSO as control. **(a)** Growth curve. **(b)** Food intake. **(c)** Fat and lean mass measured at the end of 15th week. Data represent mean $\pm$ SEM ($n=5$), * $p < 0.05$ compared to mice injected with DMSO.

Fig. 2. Effect of immune modulator on structure of adipose tissue and mRNA levels of inflammatory genes. Mice were sacrificed at the end of 15-week treatment. White and brown adipose tissues were harvested and processed. **(a)** Histological structure of white adipose tissues from treated and control mice. **(b)** Histological structure of brown adipose tissue from treated and untreated control mice. Scale bars represent 100 μ m. Relative mRNA levels of genes in white **(c)**, and brown adipose tissues **(d)**. Each data point represents the mean $\pm$ SEM (n=5), * p < 0.05 compared with mice injected with DMSO. Arrows point to crown like structure.

Fig. 3.

CsA and FTY720 suppress HFD-induced hepatic fat accumulation. Mice were sacrificed at the end of experiment and liver samples were freshly collected and processed. (a) Representative images of liver samples with H&E staining. (b) Representative images of liver samples with Oil Red O staining. Scale bars represent 100 μ m. (c) Serum levels of triglyceride (TG), total cholesterol and free fatty acid (FFA), * $p < 0.05$ compared to mice injected with DMSO.

Fig. 4. Effect of CsA and FTY720 on hepatic gene expression in the liver. RT-PCR analysis was performed to evaluate relative mRNA levels of selected genes including (a) lipid metabolism genes of *Fas*, *Acc1*, *Scd1*, and *G6p*, and (b) macrophage and T cell marker genes of *F4/80*, *Il-6*, *Tnf-α*, and *Mcp-1*. Each data point represents mean $\pm$ SEM (n=5), * p < 0.05 compared to mice injected with DMSO.

Fig. 5.

CsA and FTY720 improve insulin sensitivity in mice fed an HFD. **(a)** Time-dependent blood glucose levels following an injection of glucose (1.5 g/kg). **(b)** Area under the curve from glucose tolerance test of **(a)**. **(c)** Time-dependent glucose concentration in blood upon injection of insulin (0.75 U/kg). **(d)** Serum insulin levels. Each data point represents mean $\pm$ SEM (n=5), * p < 0.05 compared to mice injected with DMSO.

Table 1

Primer Sets for Real Time PCR Analysis of Gene Expression

Gene name	Forward sequence (F)	Reverse sequence (R)
<i>F4/80</i>	TGACTCACCTTGTGGTCCTAA	CTTCCCAGAATCCAGTCTTTCC
<i>Il-6</i>	TAGTCCTTCCTACCCCAATTCC	TTGGTCCTTAGCCACTCCTTC
<i>Tnf-α</i>	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
<i>Mcp-1</i>	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTACGGGT
<i>Cd68</i>	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTGTGA
<i>Fas</i>	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
<i>Acc-1</i>	ATGGGCGGAATGGTCTCTTTC	TGGGGACCTTGTCTTCATCAT
<i>Scd-1</i>	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
<i>G6p</i>	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA