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JNK inhibition by SP600125 Attenuates Trans-10, Cis-12 Conjugated Linoleic Acid- Mediated Regulation of Inflammatory and Lipogenic Gene Expression

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Abstract

Supplementation with a mixture of *trans*-10, *cis*-12 (t10,c12) and *cis*-9, *trans*-11 (c9,t11) isomers of conjugated linoleic acid (CLA), or t10,c12 CLA alone, reduces body weight and fat deposition in animals and some humans. However, these anti-obesity actions of t10,c12 CLA are routinely accompanied by increased markers of inflammation and insulin resistance. Thus, we examined the extent to which blocking c-Jun NH2-terminal kinase (JNK) signaling using the JNK inhibitor SP600125 attenuated markers of inflammation and insulin resistance in primary human adipocytes treated with t10,c12 CLA. SP600125 attenuated t10,c12 CLA-mediated phosphorylation of cJun and increased protein levels of activating transcription factor (ATF) 3, two downstream targets of JNK. SP600125 attenuated t10,c12 CLA-mediated induction of inflammatory genes, including interleukin (IL)-6, IL-8, IL-1 β , ATF3, monocyte chemoattractant protein (MCP)-1, and cyclooxygenase-2. Consistent with these data, SP600125 prevented t10,c12 CLA-mediated secretion of IL-8, IL-6, and MCP-1. SP600125 prevented t10,c12 CLA suppression of lipogenic genes including peroxisome proliferator activated receptor gamma, liver $\times$ receptor, sterol regulatory element binding protein, acetyl-CoA carboxylase, and stearoyl-CoA desaturase. Additionally, SP600125 blocked t10,c12 CLA-mediated induction of suppressor of cytokine synthesis-3 and suppression of adiponectin and insulin-dependent glucose transporter 4 mRNA levels. Collectively, these data suggest that JNK signaling plays an important role in t10,c12 CLA-mediated regulation of inflammatory and lipogenic gene expression in primary cultures of human adipocytes.

Keywords

conjugated linoleic acid; adipocytes; JNK; cJun; inflammation; lipogenic genes

Introduction

Obesity is a global health issue with ~ 500 million people classified as obese and 1.5 billion overweight in 2008, including 43 million children under the age of five reported in 2010 [1]. One potential strategy for reducing adiposity is consumption of conjugated linoleic acid (CLA), unsaturated fatty acids found in ruminant meats and dairy products, or in dietary

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supplements and fortified foods. Conjugated linoleic acid refers to a group of conjugated octadecadienoic acid isomers derived from linoleic acid, a fatty acid that contains 18 carbons and 2 double bonds in cis configuration at the 9th and 12th carbons (i.e., *cis*-9, *cis*-12 octadecadienoic acid). Microbes in the gastrointestinal tract of ruminant animals convert linoleic acid into different isoforms of CLA through biohydrogenation. This process changes the position and configuration of the double bonds, resulting in a single bond between one or both of the two double bonds (i.e., *cis*-9, *trans*-11 (c9,t11) or *trans*-10, *cis*-12 (t10,c12) octadecadienoic acid). Consuming a mixture of c9,t11 and t10,c12 CLA isomers, or t10,c12 CLA alone, reduces body fat mass in rodents, particularly mice, and some humans [reviewed in 2]. However, the isomer-specific mechanism by which CLA reduces adiposity is unclear. Furthermore, a number of clinical studies report potential side effects of CLA supplementation including increased levels of markers of inflammation (e.g., inflammatory cytokines, chemokines, or prostaglandins), insulin resistance, hyperlipidemia, and lipodystrophy [3–7]. These anti-obesity and adverse side effects of CLA appear to be due primarily to the t10,c12 isomer. In contrast, the c9,t11 isomer appears to have anti-inflammatory and anti-diabetic properties without reducing body weight [8].

We have demonstrated that t10,c12 CLA reduces glucose and fatty acid uptake and triglyceride content in cultures of human adipocytes, in part, by activating extracellular signal-related kinase (ERK) [9] and nuclear factor kappa B (NFκB) [10]. These *in vitro* data have been confirmed *in vivo* [11]. Activated NFκB [12–14] and ERK [15–17] induce markers of inflammation and antagonize peroxisome proliferator activated receptor (PPAR)γ activity, thereby causing insulin resistance. However, the extent to which t10,c12 CLA activates other kinases or transcription factors that impact inflammatory signaling, insulin sensitivity, and triglyceride content in human adipocytes, and their mechanism of action, are unclear.

We recently demonstrated that t10,c12 CLA increased the phosphorylation levels of c-Jun NH2-terminal kinase (JNK) and downstream targets cJun and activating transcription factor (ATF3), members of the redox-sensitive transcription factor activating protein-1 (AP-1), that induce inflammatory gene transcription [18–19]. c-Jun NH2-terminal kinase activation is known to enhance inflammation and insulin resistance associated with obesity, and lack of JNK1 or JNK2 reduces body fat and improves insulin sensitivity *in vivo* [20–21] and *in vitro* [22]. However, the role of JNK in activating cJun or ATF3 in CLA-treated cultures and the extent to which this activation regulates inflammatory and lipogenic gene expression has not been investigated.

Based on these data, we hypothesized that JNK plays an important role in t10,c12 CLA-mediated activation of AP-1 and induction of inflammatory genes and suppression of lipogenic genes. To test this hypothesis, we employed the chemical JNK1-3 inhibitor, SP600125. By using this inhibitor, we demonstrate that JNK is involved in the regulation of t10,c12 CLA-induced inflammatory signaling, and suppression of gene markers for adipogenesis, lipogenesis, and insulin signaling in cultures of newly-differentiated human adipocytes. Therefore, JNK may be an important target for preventing 10,12 CLA-mediated inflammation.

Material and Methods

Materials

All cell culture ware were purchased from Fisher Scientific (Norcross, GA). Lightning Chemiluminescence Substrate was purchased from Perkin Elmer Life Science (Boston, MA). Immunoblotting buffers and precast gels were purchased from Invitrogen (Carlsbad, CA). Adipocyte media was purchased from Zen Bio (Research Triangle Park, NC). The

Nuclear Extract Kit was purchased from Active Motif (Carlsbad, CA). Polyclonal antibodies for anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ATF3 and monoclonal antibody for anti-PPAR γ were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-total and anti-phospho (P) JNK (Thr183/Try185) and P-cJun (Ser63) antibodies were purchased from Cell Signaling Technologies (Beverly, MA). Anti-nucleoporin was purchased from BD transduction laboratories (San Jose, CA). Hyclone fetal bovine serum was purchased from Fisher Scientific. Isomers of CLA (+98% pure) were purchased from Matreya (Pleasant Gap, PA). The cell permeable selective JNK1-3 inhibitor SP600125 (#420119; Anthra[1,9-*cd*]pyrazol-6(2*H*)-one, 1,9-pyrazoloanthrone; JNKII) was purchased from EMD Chemicals (Gibbstown, NJ). This inhibitor of JNK1-3 is competitive with respect to ATP, and has over a 300-fold greater selectivity for JNK compared to other mitogen-activated protein kinase (MAPK) including ERK and p38 [23], and specifically inhibits the phosphorylation of cJun serine residues 63 and 67 [24]. All other reagents and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

Culturing of human primary adipocytes

Abdominal white adipose tissue was obtained with consent from the Institutional Review Boards at the University of North Carolina at Greensboro and the Moses Cone Memorial Hospital, during elective abdominoplasty of non-diabetic Caucasian and African American females between the ages of 20–50 years old with a body mass index ≤ 32.0 . These selection criteria allow for reduced variation in gender, age, and obesity status. Tissue was digested using collagenase; stromal vascular cells were isolated as previously described [9]. Stromal vascular cells were differentiated with adipocyte media (AM-1) containing 1 μ M rosiglitazone and 250 μ M 1-methyl-3-isobutylxanthine for 3 d, which yielded cultures containing ~30–50% adipocytes. On days 6–12, cells were pretreated with 5, 20, or 80 μ M SP600125 JNK inhibitor dissolved in DMSO for 30 min, and subsequently treated with 50 μ M t10,c12 CLA or bovine serum albumin (BSA) vehicle control for 12–24 h depending on the experimental outcome measured. All cultures were normalized to contain the same amount of BSA and DMSO vehicles. Each independent experiment was repeated at least twice using a mixture of cells from three subjects, unless otherwise indicated.

Fatty acid preparation

t10,c12 CLA was delivered as a free acid complexed to 7.5% fatty acid-free BSA at a 4:1 molar ratio as previously described [9].

RNA isolation and PCR

Total RNA was isolated from the cultures using Tri Reagent purchased from Molecular Research Center (Cincinnati, OH), according to manufacturer's protocol. For quantitative real time PCR, 2.0 μ g total RNA was converted into first strand cDNA using Applied Biosystems High-Capacity cDNA Archive Kit (Foster City, CA). Real time PCR was performed in an Applied Biosystems 7500 FAST Real Time PCR System using Taqman Gene Expression Assays. To account for possible variation in cDNA input or the presence of PCR inhibitors, the endogenous reference gene GAPDH was simultaneously quantified for each sample, and these data normalized accordingly. The Relative Standard Curve Method using seven, two-fold dilutions ranging from 100 – 1.56 ng RNA was used to check primer efficiency and linearity of each transcript according to Applied Biosystem's Guide to Performing Relative Quantification of Gene Expression Using Real-Time Quantitative PCR.

Nuclear and cytosolic separation

Nuclear and cytosolic cellular fractions were prepared using a commercially available kit from Active Motif as previously described [10].

Immunoblotting

Immunoblotting using 20 μ g of protein per lane was conducted using 4–12% NuPage precasted gels (Invitrogen) as previously described [10]. Briefly, PVDF membranes were blocked with 5% milk in TBST for 1 h and washed 3 $\times$ in TBST for 5 min. Blots were incubated overnight at 4°C with primary antibodies targeting P-JNK, P-cJun, total cJun, and ATF3 at a dilution of 1:1000, and subsequently incubated in the respective horseradish peroxidase-conjugated secondary antibody at a dilution of 1:5000 at room temperature for 1 h. Primary and secondary antibodies targeting GAPDH were used at a 1:5000 dilution. Primary and secondary antibodies targeting PPAR γ were used at dilutions 1:200 and 1:2000, respectively. After washing, blots were treated with chemiluminescence reagent for 1 min and film was exposed using a SRX-101A Konica Minolta film developer. Densitometry was performed using a Kodak Image Station 440 CF by Perkin Elmer and Kodak Molecular Imaging Software Version 4.0.

Secretion of IL-6, IL-8, and monocyte chemoattractant protein (MCP)-1

The concentrations of IL-6, IL-8, and MCP-1 were determined using the BioPlex® Suspension Array System from Bio-Rad (Hercules, CA) following the manufacturer's protocol. Briefly, media was collected from cultures that were pretreated with 5, 20, or 80 μ M SP600125 for 30 min, and subsequently treated with 50 μ M t10,c12 CLA or BSA for 24 h. The media was centrifuged at 13,200 $\times$ g for 10 min at 4°C to clear the samples of any precipitate. Samples and standards were run in duplicate. Based on the manufacturer's report 'Bio-Plex Pro Human Cytokine, Chemokine, and Growth Factor Assays - Bulletin 5828,' the intra-assay % CVs for IL-6, IL-8, and MCP-1 are 7, 9, and 9%, respectively. The inter-assay % CVs are 11, 4, and 7%, respectively.

Statistical analyses

Data are expressed as the means $\pm$ S.E. Data were analyzed using one-way analysis of variance followed by Tukey's-HSD tests for each pair for multiple comparisons. Differences were considered significant if $p < 0.05$. All analyses were performed using JMP IN, Version 9 Software (SAS Institute, Cary, NC).

Results

The JNK inhibitor SP600125 attenuates t10,c12 CLA-mediated activation of cJun and ATF3

We previously demonstrated that treatment of newly-differentiated human adipocytes with 50 μ M t10,c12 CLA, but not c9,t11 CLA, for 6 h increased phosphorylation levels of JNK and cJun, which was sustained for 24 h when compared to vehicle (BSA)-treated cultures in total cell extract [18–19]. However, a direct role for JNK in the activation of cJun and upregulation of inflammatory genes in response to t10,c12 CLA treatment has not been determined. In order to implicate a role for JNK in the activation of cJun, we examined JNK and cJun phosphorylation in cytosol and nuclear fractions after 6, 12, and 24 h of treatment with t10,c12 CLA. Phosphorylation of JNK was increased at 6 h and was sustained at 24 h in the cytosolic fraction, and increased in the nuclear fraction at 12 and 24 h with t10,c12 CLA. Interestingly, t10,c12 CLA increased total cJun levels compared to the BSA control in the nuclear fraction at all time points. Consistently, cJun phosphorylation by t10,c12 CLA was detected almost exclusively in the nuclear fraction at all time points (Fig. 1a). Based on the robust t10,c12 CLA-mediated activation of JNK and cJun, we investigated the extent to

which pretreatment with the JNK inhibitor SP600125 blocked t10,c12 CLA--mediated phosphorylation of cJun after 12 h of treatment. Concentrations of SP600125 ranging between 5–80 μ M were chosen based on studies using Jurkat T cells in which the IC₅₀ for blocking cJun phosphorylation was 10 μ M and using CD4⁺ cells isolated from human peripheral blood mononuclear cells in which the IC₅₀ for blocking cyclooxygenase (COX)-2 and tumor necrosis factor alpha expression was 5 and 10 μ M, respectively [23]. SP600125 suppressed t10,c12 CLA-mediated phosphorylation of cJun and increase in the protein levels of ATF3, an AP-1 family member, in total cell lysates (Fig. 1b). These data suggest that JNK is involved in t10,c12 CLA-mediated cJun activation.

SP600125 attenuates t10,c12 CLA induction of inflammatory genes

Next, we determined the extent to which SP600125 blocked t10,c12 CLA-induced inflammatory gene expression. Pretreatment of cultures with SP600125 attenuated t10,c12 CLA-induction of IL-8, IL-6, IL-1 β , MCP-1, COX2, and ATF3 (Fig. 2a). Consistent with these data, SP600125 attenuated t10,c12 CLA-mediated secretion of IL-8, IL-6, and MCP-1 (Fig. 2b). Collectively, these data demonstrate that SP600125 suppresses t10,c12 CLA-mediated induction of inflammatory gene expression and protein secretion.

SP600125 blocks t10,c12 CLA suppression of lipogenic genes

Inflammatory transcription factors such as NF κ B and AP-1 in concert with MAPKs like ERK have been shown to inhibit lipogenic gene expression, in part, by suppressing PPAR γ activity [12–17]. Due to the hypothesized role of JNK in regulating t10,c12 CLA-induced inflammation, we investigated the impact of SP600125 on t10,c12 CLA-mediated suppression of lipogenic gene expression and PPAR γ protein levels. Consistent with our hypothesis, SP600125 blocked t10,c12 CLA-mediated suppression of the expression of transcription factors that regulate lipid metabolism (i.e., PPAR γ / α , sterol regulatory element binding protein SREBP)-1c, liver $\times$ receptor (LXR) α) and several of their downstream targets (i.e., acetyl-CoA carboxylase (ACC)-1, stearoyl-CoA desaturase (SCD)-1) in a concentration-dependent manner (Fig. 3a). Additionally, 20 μ M SP modestly prevented a CLA-mediated decrease in PPAR γ protein levels (Fig. 3b). Taken together, these data suggest that JNK activity regulates t10,c12 CLA-mediated suppression of lipogenic genes, which may contribute to the ability of t10,c12 CLA to decrease the triglyceride levels in adipocytes.

SP600125 prevents t10,c12 CLA-mediated regulation of genes involved in insulin signaling

We previously demonstrated that t10,c12 CLA-induced inflammation leads to a suppression of insulin signaling and sensitivity [9,10,18,19]. Therefore, we determined the extent to which JNK impacted the expression of genes associated with insulin signaling. SP600125 attenuated t10,c12 CLA-mediated induction of suppressor of cytokine synthesis (SOCS)-3, a protein reported to cause insulin resistance (Fig. 4). Consistent with these data, t10,c12 CLA-mediated suppression of insulin-dependent glucose transporter (GLUT) 4 and adiponectin mRNA levels, which are positively associated with insulin sensitivity, was attenuated by SP600125 in a concentration-dependent manner (Fig. 4). Further studies are needed to confirm a role for JNK in CLA-mediated suppression of insulin-stimulated glucose uptake.

Discussion

Consistent with our hypothesis, t10,c12 CLA-mediated activation of cJun and ATF3 were attenuated by the chemical JNK inhibitor, SP600125. SP600125 also blocked t10,c12 CLA-induced inflammatory gene expression and cytokine secretion, and suppression of lipogenic genes and markers of insulin signaling. Taken together, these data suggest that JNK plays a

role in t10,c12 CLA-mediated induction of markers of inflammation and insulin resistance in cultures of human adipocytes. However, due to the potential lack of specificity of chemical inhibitors (i.e., they can inhibit other kinases including ERK and p38, albeit at much lower selectivities [23]), JNK gene silencing experiments are needed to confirm these results. Knockdown experiments are also needed to determine the extent to which JNK signaling impairs insulin-stimulated glucose uptake in t10,c12 CLA-treated cultures, because such longer-term studies were not possible with SP600125 (data not shown).

Treatment times differed based on the experimental outcome measured. Historically, we have shown in our primary cultures of newly differentiated primary human adipocytes that t10,c12 CLA increases phosphorylation of MAPKs between 6–24 h, induces inflammatory gene expression and protein secretion between 12–48 h, decreases adipogenic gene expression from 18–72 h, and PPAR γ protein from 24–48 h [9,10, 18, 19, 32, 33]. Therefore, we chose a 12 h time point to examine JNK phosphorylation, 18 h for inflammatory gene expression, 24 h for cytokine/chemokine secretion, and 24 h for adipogenic, lipogenic, and insulin-sensitizing gene expression. The timing of these events fits our hypothesis that JNK phosphorylation occurs prior to t10,c12 CLA-mediated induction and secretion of inflammatory proteins, leading to the suppression of adipogenic/lipogenic gene and protein levels.

c-Jun NH2-terminal kinase-mediated activation of cJun results in induction of several inflammatory genes including IL-8, IL-6, and COX2, and also genes involved in cell death or apoptosis. For example, SP600125 or RNA interference of JNK in 3T3-L1 adipocytes prevented free fatty acid-induced MCP-1 expression [26]. Consistent with these data, we showed that inhibiting JNK with the chemical inhibitor SP600125 prevented t10,c12 CLA-mediated inflammatory gene expression and protein secretion (Fig. 2). This prevention was accompanied by an increase in the expression lipogenic and insulin sensitizing genes and PPAR γ protein levels that promote lipogenesis or insulin signaling (Fig. 3).

One possible explanation for these data is that by inhibiting JNK activity, t10,c12 CLA was unable to decrease PPAR γ activity, which drives the expression of genes that promote glucose and fatty acid uptake and triglyceride synthesis and deposition in adipocytes. Indeed, PPAR γ 2 activity is regulated by phosphorylation [27]. Notably, phosphorylation at serine residue 112 by ERK or JNK has been reported to decrease PPAR γ activity by ubiquitination and proteasome degradation [28], and by decreasing its ligand-dependent and ligand-independent transactivating functions [15, 29–31]. Consistent with this hypothesis, we have shown that t10,c12 CLA increases PPAR γ phosphorylation prior to reducing PPAR γ protein levels. Furthermore, supplementation with Rosiglitazone, a PPAR γ agonist [32], or resveratrol, an anti-inflammatory polyphenol [33], attenuates delipidation by t10,c12 CLA. Thus, inhibiting t10,c12 CLA-mediated JNK signaling may increase PPAR γ activity, thereby increasing the expression of lipogenic and insulin-signaling genes. Consistent with this hypothesis, SP600125 supplementation of t10,c12 CLA-treated cultures increased the expression of lipogenic and insulin-signaling genes compared to cultures treated with t10,c12 CLA alone (Fig. 3).

We previously showed using the ERK inhibitor U0126 that ERK activation by t10,c12 CLA is one mechanism by which t10,c12 CLA suppresses lipogenic gene expression and insulin-stimulated glucose uptake [9]. Therefore, it is tempting to speculate that t10,c12 CLA antagonizes PPAR γ activity via activation of MAPK like ERK and JNK, thereby inhibiting PPAR γ target genes. In support of this hypothesis, knockdown of JNK1, but not JNK2, increased basal and troglitazone-stimulated PPAR γ reporter activity [34].

c-Jun NH₂-terminal kinase deficiency in animals on a high-fat diet protects them from developing insulin resistance [20]. Similarly, mitochondrial dysfunction-induced insulin resistance in 3T3-L1 adipocytes is prevented by knockdown of JNK1 [35]. Several reports show that JNK directly phosphorylates insulin receptor substrate (IRS)-1 at serine 307, thus inactivating insulin receptor signaling. However, we did not observe any effects of t10,c12 CLA or SP600125 on IRS-1 ser 307 phosphorylation (data not shown), in spite of SP600125 blocking t10,c12 CLA induction of SOCS-3, a protein reported to phosphorylate ser 307 on IRS-1. Nevertheless, t10,c12 CLA-mediated suppression of the mRNA levels adiponectin and GLUT4, proteins associated with insulin sensitivity, was completely prevented by SP600125 (Fig. 4).

Taken together, these data suggest that JNK may be an important target for preventing t10,c12-CLA mediated inflammation. Further studies are needed to confirm a role for JNK in t10,c12 CLA-mediated insulin resistance. RNA interference studies targeting JNK are also needed to confirm these data. Future research will also focus on identifying upstream activators of JNK and ERK and potential mechanisms by which these MAPK pathways are linked to insulin resistance and suppression of lipogenesis in adipocytes.

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Abbreviations

The abbreviations used are

ACC	acetyl-CoA carboxylase
AP-1	activator protein
apm-1	adiponectin
ATF	activating transcription factor 3
BMI	body mass index
BSA	bovine serum albumin
c9,t11 CLA	<i>cis</i> -9, <i>trans</i> -11 conjugated linoleic acid
t10,c12 CLA	<i>trans</i> -10, <i>cis</i> -12 conjugated linoleic acid
COX	cyclooxygenase
DEX	dexamethasone
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FA	fatty acid
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GLUT4	insulin-dependent glucose transporter 4
HBSS	Hanks balanced salt solution
IBMX	1-methyl-3-isobutylxanthine

IL	interleukin
IRS	insulin receptor substrate
ISR	integrated stress response
JNK	c-Jun-NH ₂ -terminal kinase
LXR	liver × receptor
MAPK	mitogen-activated protein kinase
MCP	monocyte chemoattractant protein
MEK	mitogen-activated protein kinase kinase
NFκB	nuclear factor kappa B
PPAR	peroxisome proliferator activated receptor
SCD	stearoyl-CoA desaturase
SOCS	suppressor of cytokine synthesis
SREBP	sterol regulatory element binding protein
SV	stromal vascular
TG	triglyceride
TZD	thiazolidinedione

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Fig. 1.

(a) Cultures of newly-differentiated human adipocytes were treated with BSA vehicle or 50 μ M t10,c12 CLA for 6, 12, or 24 h. Nuclear and cytosolic fractions were prepared using the Nuclear Extract Kit from Active Motif and analyzed for the determination of the protein levels of P-JNK, JNK, P-cJun, cJun, GAPDH, and nucleoporin. (b) Cultures were pretreated for 30 min with 5, 20, or 80 μ M SP600125 (SP) followed by a 12 h treatment with BSA vehicle or 50 μ M t10,c12 CLA. Subsequently, total cell lysates were harvested for the determination of the protein levels of P-cJun, cJun, ATF3, and GAPDH. Data are representative of two (a) or three (b) independent experiments.

Fig. 2.

(a) Cultures of newly-differentiated human adipocytes were pretreated with 5, 20, or 80 μ M SP600125 (SP) for 30 min, followed by treatment with BSA vehicle or 50 μ M t10,c12 CLA for 18 h (a) or 24 h (b). Subsequently, RNA was harvested and the mRNA levels of IL-8, IL-6, IL-1 β , MCP-1, ATF3, and COX-2 were measured by real time qPCR and normalized to GAPDH endogenous control. Means ($\pm$ SE; n=2–3 (a) or n=4 (b)) not sharing a lower case letter differ significantly ($p < 0.05$). (b). Media were collected and analyzed for IL-8, IL-6, and MCP-1 levels using the BioRad Multiplex System. Data are representative of two (b) or three (a) independent experiments.

Fig. 3.

Cultures of newly-differentiated human adipocytes were pretreated with 5, 20, or 80 μ M SP600125 (SP) for 30 min, followed by treatment with BSA vehicle or 50 μ M t10,c12 CLA for 24 h. **(a)** RNA was harvested and the mRNA levels of PPAR γ , PPAR α , LXR α , SREBP-1c, ACC-1, and SCD-1 were measured using real time qPCR and normalized to GAPDH endogenous control. Means ($\pm$ SE; $n=2-3$) not sharing a lower case letter differ significantly ($p<0.05$). **(b)** Cultures were harvested for the determination of the protein levels of PPAR γ and GAPDH. PPAR γ levels were quantified by densitometry and normalized to the loading control, GAPDH. Densitometry values are expressed as % of BSA vehicle control. Data are representative of two **(b)** or three **(a)** independent experiments.

Fig. 4.

Cultures of newly-differentiated human adipocytes were pretreated with 5, 20, or 80 μM SP600125 (SP) for 30 min, followed by treatment with BSA vehicle or 50 μM t10,c12 CLA for 24 h. Subsequently, RNA was harvested and the mRNA levels of SOCS-3, GLUT4, and apm1 were measured by real time qPCR and normalized to GAPDH endogenous control. Means (+SE; n=2–3) not sharing a lower case letter differ significantly ($p < 0.05$).