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Oleic Acid Attenuates *trans*-10, *cis*-12 Conjugated Linoleic Acid-Mediated Inflammatory Gene Expression in Human Adipocytes

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Abstract

The weight loss supplement conjugated linoleic acid (CLA) consists of an equal mixture of *trans*-10, *cis*-12 (10,12) and *cis*-9, *trans*-11 (9,11) isomers. However, high levels of mixed CLA isomers, or the 10,12 isomer, causes chronic inflammation, lipodystrophy, or insulin resistance. We previously demonstrated that 10,12 CLA decreases de novo lipid synthesis along with the abundance and activity of stearoyl-CoA desaturase (SCD)-1, a delta-9 desaturase essential for the synthesis of monounsaturated fatty acids (MUFA). Thus, we hypothesized that the 10,12 CLA-mediated decrease in SCD-1, with the subsequent decrease in MUFA, was responsible for the observed effects. To test this hypothesis, 10,12 CLA-treated human adipocytes were supplemented with oleic acid for 12 h to 7 d, and inflammatory gene expression, insulin-stimulated glucose uptake, and lipid content were measured. Oleic acid reduced inflammatory gene expression in a dose-dependent manner, and restored the lipid content of 10,12 CLA-treated adipocytes without improving insulin-stimulated glucose uptake. In contrast, supplementation with stearic acid, a substrate for SCD-1, or 9,11 CLA did not prevent inflammatory gene expression by 10,12 CLA. Notably, 10,12 CLA impacted the expression of several G-protein coupled receptors that was attenuated by oleic acid. Collectively, these data show that oleic acid attenuates 10,12 CLA-induced inflammatory gene expression and lipid content, possibly by alleviating cell stress caused by the inhibition of MUFA needed for phospholipid and neutral lipid synthesis.

Keywords

conjugated linoleic acid; adipocytes; oleic acid; inflammatory gene expression; stearoyl-CoA desaturase; monounsaturated fatty acids; G-protein receptors

Introduction

Losing body fat without reducing food intake or increasing physical activity is attractive to many who want to lose weight. One approach is consumption of conjugated linoleic acid (CLA), a fatty acid found in dairy products and ruminant meats and in supplements and fortified foods marketed worldwide for weight loss. Indeed, a meta-analysis of 18 clinical studies revealed that adults that consumed daily an average of 3.2 g of an equal mixture of two CLA isomers, *cis*-9, *trans*-11 (9,11) and *trans*-10, *cis*-12 (10,12) lost approximately 0.2 lb of body fat per week without changing food intake [1]. Intriguingly, mice consuming

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higher amounts of CLA than humans lose body fat more rapidly, but may concurrently develop side effects including chronic inflammation, insulin resistance, or lipodystrophy [2].

Only the 10,12 isomer reduces adiposity or delipidates adipocytes. However, at high doses 10,12 CLA alone and an equal mixture of 10,12 and 9,11 CLA has been reported to cause inflammation, lipodystrophy, or insulin resistance in mice [2] and human subjects [3, 4]. In contrast, the 9,11 isomer alone has anti-inflammatory and adipogenic properties, and improves insulin sensitivity in mice [5, 6]. Proposed antiobesity mechanisms of 10,12 CLA include regulation of 1) energy metabolism, 2) adipogenesis, 3) lipid metabolism, 4) inflammation, and 5) adipocyte apoptosis [reviewed in 7]. However, direct linkages of these potential mechanisms to body fat loss, especially inflammation, are unclear.

One potential mechanism by which CLA decreases adiposity is by repressing the expression or activity of lipogenic enzymes such as stearoyl-CoA desaturase-1 (SCD-1), the rate-limiting enzyme required for the synthesis of monounsaturated fatty acids (MUFA), thereby reducing the synthesis of phospholipids and neutral lipids. Such reductions in these lipids could cause cell stress given their important role in regulating membrane function and eicosanoid synthesis. Consistent with this hypothesis, 10,12 CLA alone or a mixture of 10,12 and 9,11 CLA decreases the levels of MUFA in rodents [8, 9], 3T3L1 adipocytes [10], and primary human adipocytes [11, 12]. We also recently found in primary human adipocytes that 1) 10,12 CLA decreased the expression of SCD-1 and of two transcription factors that regulate SCD-1 transcription, sterol regulatory element binding protein (SREBP)-1C and liver X receptor (LXR) α , within 5–7 h of treatment, and 2) 10,12, but not 9,11 CLA completely ablated the protein levels of SCD-1 after 12 h of treatment [12]. However, 10,12 CLA supplementation reduced body weight in SCD-1 knockout mice, simultaneously increasing the ratio of C16:0/16:1 fatty acids and decreasing the ratio of C18:0/18:1 fatty acids [13], suggesting CLA's reduction in adiposity is independent of SCD-1.

Consequences of inhibiting SCD-1 activity include increasing the ratio of saturated fatty acids to MUFA, which is known to cause inflammation and insulin resistance [reviewed in 14]. Thus, we hypothesized that supplementing 10,12 CLA-treated cultures with the MUFA oleic acid (C18:1) would overcome this anticipated blockade of MUFA synthesis, thereby mitigating cell stress associated with deficiencies in phospholipids and neutral lipids. To test this hypothesis, we supplemented 10,12 CLA-treated primary human adipocytes with micromolar levels oleic acid for 12 h to 7 d, and measured inflammatory gene and protein expression, insulin-stimulated glucose uptake, lipid content, and the expression of several G protein receptors (GPR). To show the specificity of MUFA, we also pretreated cultures with stearic acid, a substrate for SCD-1, and 9,11 CLA. Consistent with our hypothesis, oleic acid reduced inflammatory gene expression in a dose-dependent manner, and restored the lipid content of 10,12 CLA-treated adipocytes without improving insulin-stimulated glucose uptake. In contrast, supplementation with stearic acid, a substrate for SCD-1, or 9,11 CLA did not prevent increased inflammatory gene expression by 10,12 CLA. Lastly, 10,12 CLA affected the expression of several GPR, cell surface receptors that respond to extracellular stimuli including free fatty acids (FFA), which was attenuated by oleic acid. Collectively, these data show that oleic acid supplementation attenuates 10,12 CLA-induced inflammatory gene expression and lipid content, possibly by alleviating cell stress caused by the inhibition of MUFA needed for phospholipid and neutral lipid synthesis.

Materials and Methods

Materials

All cell cultureware and Hyclone fetal bovine serum were purchased from Fisher Scientific. Adipocyte medium (AM-1) was purchased from Zen-Bio. Isomers of CLA (+98% pure) were purchased from Matreya (Pleasant Gap, PA). Gene expression assays for interleukin-1 β (IL-1 β), IL-6, IL-8, cyclooxygenase-2 (COX-2), monocyte chemoattractant protein-1 (MCP-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Applied Biosystems Inc. (Foster City, CA). The polyclonal antibody for anti-GAPDH and activating transcription factor (ATF)3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-total or anti-phospho (P) JNK, ERK, I κ B α , and P-cJun antibodies were purchased from Cell Signaling Technologies (Beverly, MA). GW9508 was purchased from Tocris Bioscience (Ellisville, MO). All other chemicals and reagents were purchased from Sigma unless otherwise stated.

Cell culture model

Abdominal adipose tissue was obtained from non-diabetic Caucasian and African-American females between the ages of 20 and 50 years old with a body mass index ≥ 32.0 who had undergone elective surgery as previously described [15]. These selection criteria allow for reduced variation in gender, age, and obesity status. Institutional Review Board approval was granted through the University of North Carolina at Greensboro and the Moses H. Cone Memorial Hospital. Stromal vascular (SV) cells from human adipose tissue were isolated via collagenase digestion and subsequently grown as described previously [15]. Cells were differentiated in differentiation media [i.e., AM-1 supplemented with 250 μ M isobutylmethylxanthine and 1 μ M rosiglitazone (BRL49653; a PPAR γ agonist generously provided by Dr. Per Sauerberg at Novo Nordisk A/S, Copenhagen, Denmark)] for 3 d. Subsequently, differentiated cultures were maintained in AM-1 only for 7–14 d. These cultures contain approximately 50% adipocytes and 50% preadipocytes by day 7. CLA isomers were given at physiological levels [16, 17]. Oleic acid was given at micromolar levels given its abundance in human blood [18] and adipocytes [11]. Fatty acids were complexed to fatty acid-free (>98%) bovine serum albumin (BSA; Sigma A7030, lot #040M1649) at a 4:1 molar ratio. This BSA was chosen based on its decreased capacity to increase inflammatory gene expression compared to other BSA samples tested (unpublished data). BSA levels were normalized to the highest fatty acid treatment so that all cultures contained the same amount of BSA vehicle. Fatty acids treatments were added at the same time. Each experiment was repeated in triplicate using a mixture of cells from at least two different subjects unless otherwise indicated.

RNA isolation and real-time quantitative PCR (qPCR)

Primary human SV cells were seeded in 35-mm dishes at 0.4×10^6 cells per dish and differentiated for 3 d. The media was changed on days 3 and 6. Cells were treated on day 7 with fatty acids or BSA vehicle control. After 18 h RNA was isolated from cell cultures using the RNeasy mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol for human adipocytes. For real time qPCR, 2 μ g of total RNA was used to generate first strand cDNA using the Applied Biosystems high capacity cDNA archive kit. qPCR was performed using a 7500 Fast Real-time PCR System (Applied Biosystems) using Taqman $^{\circledR}$ Universal PCR Master Mix and Taqman gene expression assays. To account for possible variation related to cDNA input amounts or the presence of PCR inhibitors, the endogenous reference gene GAPDH was quantified simultaneously for each sample in separate wells of the same 96-well plate.

Lipid content determination

Primary human SV cells were seeded in 12-well plates at 2×10^6 cells per plate and differentiated for 3 d. The media was changed to AM-1 on days 3 and 6. Cells were treated on days 7 and 11 with fatty acids or BSA control. On day 14, media was removed and cell cultures were washed with Hank's Balanced Salt Solution (HBSS). The presence of intracellular lipid was visualized by staining the cultures with oil red O, and lipid content was quantified based on absorbance levels at 540 nm as we previously described [11].

Immunoblotting

Immunoblotting using 20 μ g of protein per lane was conducted using 4–12% NuPage precasted gels (Invitrogen) as previously described [19]. 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 I κ B α , P-cJun, P-JNK, P-ERK, total cJun, total ERK, 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. 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.

Insulin-stimulated 2-[³H]deoxy-glucose (2-DOG) uptake

Primary human SV cells were seeded in 12-well plates at 1.6×10^5 per well and differentiated for 12 d. On day 12, media were changed to serum free low glucose (5 mmol/L) media. Twenty four hours later, cultures were pretreated with fatty acids or BSA control. Culture media was removed and replaced with 0.5 mL of HBSS without or with 100 nmol/L human insulin for 10 min. After insulin preincubation, 4 nM 2-DOG (0.5 μ Ci per well) was added to each well and incubated at 37°C for 90 min. Basal and insulin-stimulated 2-DOG uptake were measured as described previously [11].

Statistical Analysis

Data are expressed as the means $\pm$ SEM. Data were analyzed using one-way analysis of variance followed by Student's *t* tests for each pair for multiple comparisons. Differences were considered significant if *p* < 0.05. All analyses were performed using JMP IN version 9.0 software (SAS Institute, Cary, NC).

Results

Oleic acid attenuates 10,12 CLA-mediated inflammatory gene expression and delipidation

In order to determine the extent to which 10,12 CLA-induced inflammatory gene expression was caused by inhibition of SCD-1-mediated MUFA synthesis [11, 12], we co-supplemented 10,12 CLA-treated primary human adipocytes with oleic acid. Oleic acid and 9,11 CLA alone had no effect on inflammatory gene expression (Fig. 1.). Consistent with our hypothesis, 10,12 CLA-mediated increases in the expression of IL-6, IL-8, COX-2, and IL-1 β were decreased in a dose-dependent manner by co-supplementation with oleic acid (Fig. 1.). However, oleic acid did not prevent 10,12 CLA-mediated activation of ERK, JNK, cJun, or I κ B α or increased abundance of ATF3 (data not shown). Given the decrease in the inflammatory response by oleic acid, we speculated that oleic acid would also attenuate 10,12 CLA-induced delipidation. Indeed, 10,12 CLA-mediated delipidation was blocked by co-supplementation with oleic acid (Fig. 2). However, oleic acid did not prevent 10,12 CLA-mediated insulin resistance (data not shown).

Stearic acid or 9,11 CLA supplementation do not attenuate 10,12 CLA-mediated inflammatory gene expression or delipidation

In order to determine the specificity of oleic acid inhibition of inflammatory gene expression in 10,12 CLA-treated cultures, primary human adipocytes were co-supplemented with stearic acid, a substrate for SCD-1 and a precursor to oleic acid. Unlike oleic acid, stearic acid alone increased the expression of IL-6, IL-8, and COX-2 (Fig. 3), consistent with the reported effects of saturated fatty acids on markers of inflammation [reviewed in 14]. Furthermore, stearic acid co-supplementation did not attenuate 10,12-mediated inflammatory gene expression (Fig. 3). In fact, the two highest levels of stearic acid exacerbated 10,12 CLA-mediated induction of inflammatory genes.

Because of the reported anti-inflammatory and anti-diabetic properties of 9,11 CLA [5], we co-supplemented 10,12 CLA-containing cultures with 9,11 CLA. Although 9,11 CLA alone did not increase inflammatory gene expression, its inclusion in the 10,12 CLA-treated cultures did not prevent 10,12 CLA-mediated inflammatory gene expression (Fig. 4) or delipidation (data not shown). These data demonstrate that the 9,11 CLA isomer does not directly prevent 10,12 CLA-mediated inflammatory gene expression or delipidation in vitro. Collectively, these data suggest that CLA-mediated inflammatory gene expression is due, in part, to lack of oleic acid, an abundant MUFA [11, 18] required by mammalian cells for the synthesis of phospholipids and neutral lipids.

Oleic acid influences the effects of 10,12 CLA on GPR expression

FFA activate FFA receptors, including G-protein-coupled receptors (GPCR) and GPR, that impact inflammatory signaling and metabolism [reviewed in 20]. Notably, activation of GPR120 has anti-inflammatory properties. Therefore, we examined the effects of 10,12 CLA in the absence and presence of oleic acid on the expression of several GPR expressed in adipocytes. Interestingly, 10,12 CLA decreased the expression of GPR120, but increased the expression of GPR56 and GPRC5A (Fig. 5a). Furthermore, the GPR40/120 agonist GW9508 attenuated 10,12 CLA-mediated increase of inflammatory gene expression and suppression of lipogenic gene expression (Fig. 5b). Intriguingly, oleic acid supplementation attenuated 10,12 CLA-mediated suppression of GPR120, and induction of GPRC5A (Fig. 5c), but not GPR56 (data not shown). Taken together, these data suggest that 10,12 CLA influences specific GPR that impact signaling pathways involved in inflammation and lipogenesis.

Discussion

Consistent with our hypothesis, oleic acid supplementation attenuated 10,12 CLA-induced inflammatory gene expression (Fig. 1) and delipidation (Fig. 2) in primary human adipocytes. In contrast, stearic acid, a substrate for SCD-1, or 9,11 CLA did not prevent 10,12 CLA-mediated increase in inflammatory gene expression (Fig. 3, 4) or delipidation (data not shown). These data are consistent with our previous findings in primary human adipocytes showing that 10,12 CLA decreased 1) de novo lipid synthesis within 24–72 h, (2) the MUFA/saturated fatty acid ratio within 24 h, 3) the mRNA and protein levels of SCD-1 within 7 h in an isomer-specific fashion, and 4) the expression of LXR α and SREBP-1c within 5 h [12]. They are also consistent with data obtained from mice [8, 9], and murine [10, 21–23] or porcine [24] (pre)adipocytes, suggesting that 10,12 CLA rapidly reduces the abundance or activity of SCD-1. Collectively, these data suggest that by reducing the abundance or levels of lipogenic transcription factors that control SCD-1 (i.e., LXR α , SREBP-1c, PPAR γ), 10,12 CLA suppresses the synthesis of MUFA needed for phospholipid and neutral lipid synthesis, storage, or metabolism. Such a scenario could conceivably cause cell stress that impairs the adipocyte's capacity to sequester, synthesize,

and store lipids. Consistent with this hypothesis, we previously demonstrated that 10,12 CLA, but not 9,11 CLA, decreased de novo lipid synthesis of triglycerides (TG), FFA, diacylglycerol, cholesterol esters, cardiolipin, phospholipids, and ceramides in human adipocytes within 3- 24 h [12].

Ntambi first proposed that 10,12 CLA-mediated inhibition of SCD-1 in mice [10, 21–22] and in humans [23] is important for its anti-lipogenic effects. SCD-1 and diacylglycerol transferase-2 (DGAT2) have been shown to co-localize in the ER and be important for TG synthesis [25]. Dietary and endogenous palmitate (C16:0) and stearate (C18:0) are desaturated by SCD-1 and channeled to DGAT2 for the final step in TG synthesis in the ER. This close association between SCD-1 and DGAT2 enhance the efficiency of TG synthesis [25]. However, 10,12 CLA-mediated reduction in body fat mass was similar in SCD-1 knockout and wild type mice, suggesting that the reduction in adiposity by 10,12 CLA is independent of SCD-1 in mice [13]. Indeed, SCD-1 is not the only lipogenic gene suppressed by 10,12 CLA, given its antagonism of the key lipogenic transcription factors LXR α , SREBP-1c, and PPAR γ [reviewed in 7].

We anticipated that, because oleic acid supplementation attenuated inflammatory gene expression, it would also decrease the activation of upstream transcription factors and mitogenactivated protein kinases (MAPK) controlling the expression of these genes. However, 10,12 CLA-mediated activation of ERK, JNK, cJun, ATF3, and NF- κ B was not inhibited by oleic acid supplementation (data not shown). Reasons for this lack of inhibition of inflammatory transcription factors and MAPK are unclear, as we have previously shown that inhibiting 10,12 CLA-mediated activation of NF- κ B [26], cJun [27], ERK [15], and JNK [19] attenuates 10,12 CLA-mediated inflammatory gene expression. Perhaps 10,12 CLA's reported increase in the typical integrated stress response [28] or atypical ER stress response [29] contributes to the induction of inflammatory gene expression independent of NF- κ B, AP-1, or MAPK.

Alternatively, oleic acid may attenuate 10,12 CLA-mediated inflammatory gene expression by impacting specific GPCR or GPR that regulate inflammatory signaling and delipidation. GPR40, 84, 119, and 120 are classically activated by long chain FFA, and GPR41 and 43 by short chain FFA. Importantly, 1) cell surface, FFA receptors [30–32] and putative GPCR [33–35] activated by FFA impact on cell signaling, 2) CLA reduces FFA transport in tumors and white adipose tissue by activating specific GPCR [36, 37], 3) CLA increased pancreatic insulin release via islet GRP40 [38], and 4) we found that impairing coupling between GPCR and Gi/o with pertussis toxin blocked 10,12 CLA's suppression of PPAR γ target gene expression and lipid metabolism in adipocytes [15].

Notably, long chain unsaturated FFA such as docosahexanoic acid activate GPR120, which is highly expressed in adipose tissue and contributes to their anti-inflammatory properties [30]. GPR120 also binds other mono- and polyunsaturated fatty acids including oleic acid [39]. Consistent with these data, we found that 1) oleic acid increased GPR120 expression (Fig. 5c), 2) 10,12 CLA decreased the expression of GPR120 (Fig. 5a,c), which was attenuated by supplementation with oleic acid (Fig. 5c), 3) the GPR40/120 agonist GW9508 attenuated 10,12 CLA-mediated inflammatory gene expression and suppression of lipogenic gene expression (Fig. 5b), 4) oleic acid supplementation inhibited 10,12 CLA-mediated suppression of GPR120 (Fig. 5c), and 5) 10,12 CLA had no effect on the expression of GPR40 (data not shown), which had very low levels of expression in primary human adipocytes. These data suggest that 10,12 CLA's pro-inflammatory effects may be linked upstream to its inhibition of GPR120. Studies on the activity of GPR120 in cells treated with 10,12 CLA are needed to test this hypothesis.

We also measured the expression of GPR56 and GPRC5A, members of the non-classical adhesion [40] and tumor suppressor [41] families reported to be expressed in inflamed tissues [42], respectively, that we identified as CLA- induced candidates in a microarray assay (unpublished data). Indeed, 10,12 CLA increased the expression of GPRC5A and GPR56 (Fig. 5a), and oleic acid supplementation decrease this response for GPRC5A (Fig. 5c). Aside from the fact that these GPR are expressed in inflamed tissues, the physiological significance of these GPR findings in primary human adipocytes is unclear at this time.

Taken together, these data demonstrate that oleic acid supplementation attenuates 10,12 CLA-induced inflammatory gene expression and delipidation in primary human adipocytes, possibly by alleviating cell stress [28, 29] caused by the inhibition of SCD-1 [11, 12]. Our previous findings showed that 10,12 CLA rapidly reduces de novo lipid synthesis proceeded by inhibition of transcriptional regulators of lipogenesis and their downstream targets, including SCD-1 [12]. Therefore, we hypothesize that these changes impair MUFA synthesis needed for phospholipid and neutral lipid accumulation, thereby delipidating human adipocytes.

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Abbreviations

AP-1	activator protein
ATF	activating transcription factor 3
BMI	body mass index
BSA	bovine serum albumin
9,11 CLA	<i>cis</i> -9, <i>trans</i> -11 conjugated linoleic acid
10,12 CLA	<i>trans</i> -10, <i>cis</i> -12 conjugated linoleic acid
COX	cyclooxygenase
DOG	deoxyglucose
DEX	dexamethasone
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FFA	free fatty acid
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPRC	G-protein coupled receptors
GPR	G-protein receptors
JNK	c-Jun-NH2-terminal kinase
LXR	liver X receptor
MAPK	mitogen-activated protein kinase
MCP	monocyte chemoattractant protein
MEK	mitogen-activated protein kinase kinase

MUFA	monounsaturated fatty acids
NF-κB	nuclear factor kappa B
PPAR	peroxisome proliferator activated receptor
SCD	stearoyl-CoA desaturase
SREBP	sterol regulatory element binding protein
SV	stromal vascular
TG	triglyceride

References

1. Whigham LD, Watras AC, Schoeller DA. Efficacy Of Conjugated Linoleic Acid For Reducing Fat Mass: A Meta-Analysis In Humans. *Am J Clin Nutr.* 2007; 85:1203–1211. [PubMed: 17490954]
2. Poirier H, Niot I, Clement L, Guerre-Millo M, Besnard P. Development Of Conjugated Linoleic Acid (CLA)-Mediated Lipoatrophic Syndrome In The Mouse. *Biochimie.* 2005; 87:73–79. [PubMed: 15733740]
3. Risérus U, Arner P, Brismar K, Vessby B. Treatment with Dietary trans-10, cis-12 Conjugated Linoleic Acid Causes Isomer Specific Insulin Resistance in Obese Men with the Metabolic Syndrome. *Diabetes Care.* 2002; 25:1516–1521. [PubMed: 12196420]
4. Tholstrup T, Raff M, Staarup E, Lund P, Basu S, Bruun JM. An Oil Mixture with trans-10, cis-12 Conjugated Linoleic Acid Increases Markers of Inflammation and in vivo Lipid Peroxidation Compared with cis-9, trans-11 Conjugated Linoleic Acid in Postmenopausal Women. *J Nutr.* 2008; 138:1445–1451. [PubMed: 18641189]
5. Moloney F, Toomey S, Noone E, Nugent A, Allan B, Loscher CE, Roche HM. Antidiabetic Effects of cis-9, trans-11 Conjugated Linoleic Acid May be Mediated via Anti-Inflammatory Effect in White Adipose Tissue. *Diabetes.* 2007; 56:574–582. [PubMed: 17327424]
6. Halade G, Halade GV, Rahman MM, Fernandes G. Differential Effects Of Conjugated Linoleic Acid Isomers In Insulin-Resistant Female C57bl/6j Mice. *J Nutr Biochem.* 2010; 21:332–337. [PubMed: 19423318]
7. Kennedy A, Martinez K, Schmidt S, Mandrup S, Lapoint K, McIntosh M. Antiobesity Mechanisms Of Action Of Conjugated Linoleic Acid. *J Nutr Biochem.* 2010; 21:171–179. [PubMed: 19954947]
8. Martin JC, Grégoire S, Siess MH, Genty M, Chardigny JM, Berdeaux O, Juanéda P, Sébédio JL. Effects of Conjugated Linoleic Acid Isomers on Lipid Metabolizing Enzymes in Male Rats. *Lipids.* 2000; 35:91–98.
9. House RL, Cassady JP, Eisen EJ, Eling TE, Collins JB, Grissom SF, Odle J. Functional Genomic Characterization of Delipidation Elicited by trans-10, cis-12 Conjugated Linoleic Acid (t10c12-CLA) in a Polygenic Obese Line of Mice. *Physiol Genomics.* 2005; 21:351–361. [PubMed: 15888570]
10. Choi Y, Kim YC, Han YB, Park Y, Pariza MW, Ntambi JM. The trans-10, cis-12 Isomer of Conjugated Linoleic Acid Downregulates Stearoyl-CoA Desaturase-1 Gene Expression in 3T3-L1 Adipocytes. *J Nutr.* 2000; 130:1920–1924. [PubMed: 10917902]
11. Brown M, Sandberg-Boysen M, Skov S, Morrison R, Storkson J, Lea-Currie R, Pariza M, Mandrup S, McIntosh M. Isomer Specific Regulation of Metabolism and PPAR γ by Conjugated Linoleic Acid (CLA) in Human Preadipocytes. *J Lipid Res.* 2003; 44:1287–1300. [PubMed: 12730300]
12. Obsen T, Faergeman N, Chung S, Martinez K, Gobern S, Loreau L, Wabitsch L, Mandrup SM, McIntosh M. Trans-10, Cis-12 Conjugated Linoleic Acid Decreases De Novo Lipid Synthesis In Human Adipocytes. *J Nutr Biochem.* 2012; 23:580–590. [PubMed: 21775116]
13. Kang K, Miyazaki M, Ntambi JM, Pariza MW. Evidence that the Antiobesity Effect of Conjugated Linoleic Acid is Independent of Effects on Stearoyl-CoA Desaturase-1 Expression and Enzyme Activity. *Biochem Biophys Res Commun.* 2004; 315:532–537. [PubMed: 14975733]

14. Kennedy A, Martinez K, Chuang CC, LaPoint K, McIntosh M. Saturated Fatty Acid-Mediated Inflammation and Insulin Resistance in Adipose Tissue - Mechanisms of Actions and Implications. *J Nutr.* 2009; 139:1–4. [PubMed: 19056664]
15. Brown JM, Boysen MS, Chung S, Fabiyi O, Morrison RF, Mandrup S, McIntosh M. Conjugated Linoleic Acid Induces Human Adipocyte Delipidation: Autocrine/Paracrine Regulation Of MEK/ERK Signaling By Adipocytokines. *J Biol Chem.* 2004; 279:26735–26747. [PubMed: 15067015]
16. Mougios V, Matsakas A, Petridou A, Ring S, Sagredos A, Melissopoulous A, Tsigilis N, Nikolaidis M. Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. *J Nutr Biochem.* 2001; 12:585–594. [PubMed: 12031264]
17. Petridou A, Mougios V, Sagredos A. Supplementation with CLA: Isomer Incorporation into Serum Lipids and Effect on Body Fat of Women. *Lipids.* 2003; 38:805–811. [PubMed: 14577658]
18. Martinez K, Kennedy A, McIntosh M. JNK Inhibition by SP600125 Attenuates trans-10, cis-12 Conjugated Linoleic Acid-Mediated Regulation of Inflammation and Lipogenic Gene Expression. *Lipids.* 2011; 46:885–892. [PubMed: 21744278]
19. Samieri C, Fear C, Proust-Lima C, Peuchant E, Tzourio C, Stapf C, Berr C, Barberger-Gateau P. Olive oil consumption, plasma oleic acid, and stroke incidence. *Neurology.* 2011; 77:418–425. [PubMed: 21676914]
20. Oh DY, Lagakos WS. The role of G-protein-coupled receptors in mediating the effects of fatty acids on inflammation and insulin sensitivity. *Curr Opin Clin Nutr Metab Care.* 2011; 14:322–327. [PubMed: 21587066]
21. Lee K, Pariza M, Ntambi J. Conjugated Linoleic Acid Decreases Hepatic Stearoyl CoA Desaturase mRNA Expression. *Biochem Biophys Res Commun.* 1998; 248:817–821. [PubMed: 9704011]
22. Park Y, Storkson J, Ntambi J, Cook M, Sih C, Pariza M. Inhibition of Hepatic Stearoyl-CoA Desaturase Activity by trans-10, cis-12 CLA and its Derivative. *Biochem Biophys Acta.* 2000; 1486:285–292. [PubMed: 10903479]
23. Choi Y, Park Y, Pariza M, Ntambi J. Regulation of Stearoyl-CoA Desaturase Activity by trans-10, cis-12 CLA in HepG2 Cells. *Biochem Biophys Res Commun.* 2001; 284:689–693. [PubMed: 11396956]
24. Brandebourg TD, Hu CY. Isomer-specific Regulation of Differentiating Pig Preadipocytes by Conjugated Linoleic Acids. *J Anim Sci.* 2005; 83:2096–2105. [PubMed: 16100064]
25. Man WC, Miyazaki M, Chu K, Ntambi J. Co-localization of SCD1 and DGAT2: Implying Preference for Endogenous Monounsaturated Fatty Acids in Triglyceride Synthesis. *J Lipid Res.* 2006; 47:1928–1939. [PubMed: 16751624]
26. Chung S, Brown JM, Provo JN, Hopkins R, McIntosh M. Conjugated Linoleic Acid Promotes Human Adipocyte Insulin Resistance Through NFkB-dependent Cytokine Production. *J Biol Chem.* 2005; 280:38445–38456. [PubMed: 16155293]
27. Martinez K, Kennedy A, West T, Milatovic D, Aschner M, McIntosh M. Trans-10, cis-12 conjugated linoleic acid instigates inflammation in human adipocytes compared to preadipocytes. *J Biol Chem.* 2010; 285:17701–17712. [PubMed: 20353947]
28. LaRosa P, Riethoven J, Chen H, XIA Y, Zhou YL, Chen M, Miner J, Fromm M. Trans-10, cis-12 Conjugated Linoleic Acid Activates the Integrated Stress Response Pathway in Adipocytes. *Physiol Genomics.* 2007; 31:544–553. [PubMed: 17878318]
29. Ou L, Wu Y, Ip C, Meng X, Hsu Y, Ip M. Apoptosis Induced by t10,c12 Conjugated Linoleic Acid is Mediated by an Atypical Endoplasmic Reticulum Stress Response. *J Lipid Res.* 2008; 49:985–994. [PubMed: 18263853]
30. Oh DY, Talukdar SE, Bae J, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky SM. GPR120 Is An Omega-3 Fatty Acid Receptor Mediating Potent Anti-Inflammatory And Insulin-Sensitizing Effects. *Cell.* 2010; 142:687–698. [PubMed: 20813258]
31. Kotarsky K, Nilsson NE, Flodgren E, Owman C, Olde B. A Human Cell Surface Receptor Activated By Free Fatty Acids And Thiazolidinedione Drugs. *Biochem Biophys Res Commun.* 2003; 301:406–410. [PubMed: 12565875]
32. Soto-Guzman A, Robledo T, Lopez-Perez M, Salazar EP. Oleic Acid Induces Erk1/2 Activation And Ap-1 Dna Binding Activity Through A Mechanism Involving Src Kinase And Egfr

- Transactivation In Breast Cancer Cells. *Mol Cell Endocrinol*. 2008; 294:81–91. [PubMed: 18775472]
33. Briscoe CP, Tadayyon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, Ellis C, Elshourbagy NA, Goetz AS, Minnick DT, Murdock PR, Sauls HR, Shabon U, Spinage LD, Strum JC, Szekeres PG, Tan KB, Way JM, Ignar DM, Wilson S, Muir AI. The Orphan G Protein-Coupled Receptor GPR40 Is Activated By Medium And Long Chain Fatty Acids. *J Biol Chem*. 2003; 278:11303–11311. [PubMed: 12496284]
 34. Qanbar R, Bouvier M. Role Of Palmitoylation/Depalmitoylation Reactions In G-Protein-Coupled Receptor Function. *Pharmacol Ther*. 2003; 97:1–33. [PubMed: 12493533]
 35. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M. Free Fatty Acids Regulate Insulin Secretion From Pancreatic Beta Cells Through GPR40. *Nature*. 2003; 422:173–176. [PubMed: 12629551]
 36. Sauer LA, Dauchy RT, Blask DE, Krause JA, Davidson LK, Dauchy EM, Welham KJ, Coupland K. Conjugated Linoleic Acid Isomers And Trans Fatty Acids Inhibit Fatty Acid Transport In Hepatoma 7288ctc And Inguinal Fat Pads In Buffalo Rats. *J Nutr*. 2004; 134:1989–1997. [PubMed: 15284388]
 37. Hsu YC, Ip M. Conjugated linoleic acid-induced apoptosis in mouse mammary tumor cells is mediated by both G protein-coupled receptor-dependent activation of the AMP-activated protein kinase pathway and by oxidative stress. *Cellular Signaling*. 2011; 23:2013–2020.
 38. Schmidt J, Liebscher K, Merten N, Grundmann M, Mielenz J, Sauerwein H, Christiansen E, Due-Hansen M, Ulven T, Ullrich S, Gomez J, Drewke C, Kostenis E. Conjugated linoleic acids mediate insulin release through islet G protein coupled receptor FFA1/GPR40. *J Biol Chem*. 2011; 286:11890–11894. [PubMed: 21339298]
 39. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med*. 2005; 11:90–94. [PubMed: 15619630]
 40. Paavola K, Stephenson J, Ritter S, Alter S, Hall R. The N terminus of the adhesion G protein-coupled receptor GPR56 control receptor signaling activity. *J Biol Chem*. 2011; 33:28914–28921. [PubMed: 21708946]
 41. Deng J, Fukimoto J, Ye X, Men T, Van Pelt C, Chen Y, Lin X, Kadara H, Tao Q, Lotan D, Lotan R. Knockout of the tumor suppressor gene GPRC5a in mice leads to NF- κ B activation in airway epithelium and promotes lung inflammation and tumorigenesis. *Cancer Prev Res*. 2010; 3:424–436.
 42. Chiesa D, Falco M, Parolini F, Bellora F, Petretto A, Romeo E, Balsamo M, Gambarotti M, Scordamaglia F, Tabellini G, Facchetti F, Vermi W, Bottino C, Moretta A, Vitale M. GPR56 as a novel marker for identifying the CD56null CD16+ NK cell subset both in blood stream and in inflamed peripheral tissues. *Int Immun*. 2010; 22:91–100. [PubMed: 20008459]

Fig. 1.

Cultures of primary human adipocytes were treated without or with 30–300 μM oleic acid (OA), 50 μM 9,11 CLA, or 50 μM 10,12 CLA for 12 h. Subsequently, mRNA levels of IL-8, IL-6, IL-1β, COX-2, and GAPDH (load control) were measured using qPCR. Data were normalized to BSA vehicle controls (OA, 0, -CLA). Means ± S.E. (n=3–4) not sharing a common superscript (a-e) are significantly different (p<0.05). Data are representative of three independent experiments.

Fig. 2.

Cultures of primary human adipocytes were treated without or with 300 μ M oleic acid (OA), 50 μ M 10,12 CLA, or BSA vehicle (0 OA, 0 CLA) for 7 d. Subsequently, **a)** cultures were stained with oil red O and photographed using an Olympus inverted microscope with a 20x objective and **b)** the stain concentration was quantified. **b)** Means + S.E. (n=3) not sharing a common superscript (a-c) are significantly different ($p < 0.05$). Data are representative of two independent experiments.

Fig. 3.

Cultures of primary human adipocytes were treated without or with 10–100 μM stearic acid (SA), 50 μM 9,11 CLA, or 50 μM 10,12 CLA for 12 h. Subsequently, mRNA levels of IL-8, IL-6, IL-1β, COX-2, and GAPDH (load control) were measured using qPCR. Data were normalized to BSA vehicle controls (SA, 0, -CLA). Means ± S.E. (n=3–4) not sharing a common superscript (a-e) are significantly different (p<0.05). Data are representative of two independent experiments.

Fig. 4.

Cultures of primary human adipocytes were treated without or with 25–50 μM 9,11 CLA, or 10,12 CLA for 18 h. Subsequently, mRNA levels of IL-8, IL-6, IL-1 β , COX-2, and GAPDH (load control) were measured using qPCR. Data were normalized to BSA vehicle controls (–9,11, –10,12 CLA). Means $\pm$ S.E. (n=3–4) not sharing a common superscript (a–e) are significantly different ($p < 0.05$). Data are representative of three independent experiments.

Fig. 5.

Cultures of primary human adipocytes were treated without or with: **a)** 50 μ M 9,11 CLA, 10,12 CLA, or BSA vehicle for 12 h; **b)** 50 μ M 10,12 CLA or 1, 10, or 100 μ M GW9508 for 18 h; or **c)** 30–300 μ M oleic acid (OA), 50 μ M 9,11 CLA, or 50 μ M 10,12 CLA for 12 h. Subsequently, mRNA levels of candidate genes were measured using qPCR. Data were normalized to BSA vehicle controls. Means $\pm$ S.E. ($n=3-4$) not sharing a common superscript (a-e) are significantly different ($p<0.05$). Data are representative of at least two independent experiments.