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SCD1 mediates the influence of exogenous saturated and monounsaturated fatty acids in adipocytes: Effects on cellular stress, inflammatory markers and fatty acid elongation

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Abstract

Palmitate (PA), stearate (SA), palmitoleate (PMA) and oleate (OA) are among the most abundant fatty acids (FAs) in adipose tissue (AT). These FAs differentially regulate AT inflammation by altering adipocyte signalling pathways and the secretion of proinflammatory cytokines. Intracellular levels of these FAs are controlled, in part, by stearoyl-CoA desaturase 1 (SCD1). Therefore, SCD1 may have an important role mediating FA-regulation of adipocyte inflammation. Given this, we hypothesized that the influence of PA, SA, PMA and OA on inflammation and cellular stress, as well as FA metabolism, would be exacerbated with reduced SCD1 activity. Real-time RT-PCR, immunoassays, gas chromatography and western blotting were used to examine the expression and secretion of common inflammatory markers, as well as FA profiles and markers of cellular stress, in 3T3-L1 adipocytes. FA treatments differentially affected inflammatory markers and FA profiles in SCD1-inhibited adipocytes. Specifically, SA significantly increased the expression of *Ccl5* (5.3-fold) and *Mcp-1* (3.2-fold), and the secretion of IL-6 (17.8-fold) and MCP-1 (4.0-fold) in SCD1-inhibited adipocytes compared to controls. The proinflammatory effects observed with SA are particularly notable given that SCD1-inhibited adipocytes increased elongation of PA to SA, as determined using U-^{13}C-PA. The effects of PA, PMA and OA were not as substantial as those of SA, although PA did significantly increase *Ccl5* (2.7-fold) and *Mcp-1* (1.2-fold) expression in SCD1-inhibited adipocytes. None of the FAs altered markers of cellular stress. Collectively, these results emphasize the differential effects of individual FAs and highlight how SCD1 influences their regulation of adipocyte inflammation.

Keywords: Palmitate; Stearate; Palmitoleate; Oleate; Stearoyl-CoA desaturase 1; SCD1 Inhibitor; Elongase 6

1. Introduction

Obesity is considered a state of chronic low-grade inflammation and is a major worldwide health concern [1,2]. Adipose tissue (AT) hypertrophy is associated with the dysregulation of cytokine and chemokine production and secretion [1,3]. These secreted proteins play a key role in the paracrine dialogue between the various cell types comprising AT (*e.g.*, adipocytes, macrophages, endothelial cells, T-cells), which ultimately regulates numerous biological processes including insulin signalling and cellular stress [1]. As such, the regulation of inflammatory signalling pathways is critically important for overall AT metabolism. Fatty acids (FAs) are one class of compounds that are documented to regulate adipocyte inflammation [4–6]. For example, previous work by van Dijk *et al.* showed that consuming a diet high in SFAs increased proinflammatory gene expression in human AT, yet a diet high in MUFAs had an antiinflammatory effect [4]. In addition, we previously showed that the two most common SFAs, palmitate (PA) and stearate (SA), differentially regulated inflammatory signalling pathways in 3T3-L1 preadipocytes [5]. Together, the existing evidence demonstrates that individual FAs have distinct effects on AT inflammation and metabolism. This emphasizes the need to examine individual FAs in order to delineate their links with inflammation and adipocyte metabolism.

FAs that are derived from the diet or from *de novo* lipogenesis are primarily stored in adipocytes as triacylglycerols (TAGs) [3]. As such, adipocytes express a panel of genes that enable the production, metabolism and storage of FAs [7]. Stearoyl-CoA desaturase 1 (SCD1) plays a central role in FA metabolism by converting the SFAs PA and SA to the MUFAs palmitoleate (PMA) and oleate (OA), respectively [8]. SCD1 has been previously associated with alterations in cellular inflammation and stress, where primary adipocytes isolated from whole-body SCD1-deficient mice were shown to have reduced inflammation compared to wild-type adipocytes [9,10]. In addition, Malodobra-Mazur *et al.* have recently shown that SCD1 can mediate inflammation in 3T3-L1 adipocytes by regulating DNA methylation [11]. Given the purported link between SCD1 and inflammation, as well as SCD1's central role in FA metabolism, it is of interest to determine if SCD1 mediates the relationships between individual FAs and inflammation.

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FA substrates and products of SCD1 are among the most abundant FAs in AT [5,12–15]. Consequently, FA regulation of adipocyte signalling pathways may be altered when SCD1 activity is compromised. Therefore, our objective was to examine if SCD1 inhibition in adipocytes altered the influence of PA, PMA, SA and OA on lipid metabolism, cellular stress and inflammation. We hypothesized that the inflammatory and stress response to FAs (in particular SFAs) would be greater in adipocytes with compromised SCD1 activity compared to control adipocytes. Our results not only highlight the complex associations between SCD1, FAs, inflammation and adipocyte metabolism but also demonstrate how SCD1 has an important role mediating the effects of SFAs and MUFAs in adipocytes.

2. Materials and methods

2.1. Chemicals and cell culture reagents

A specific SCD1 inhibitor (CAY10566) was purchased from Cayman Chemical (Ann Arbor, MI, USA), as previously described [16]. The murine 3T3-L1 preadipocytes were obtained from ATCC (Rockville, MD, USA). Cell culture reagents including Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin (pen–strep) and 0.25% trypsin-ethylenediaminetetraacetic acid solution were purchased from Hyclone laboratories (Logan, UT, USA). Other cell culture reagents including 3-isobutyl-1-methylxanthine (IMBX), dexamethasone (Dex), human insulin, FA-free bovine serum albumin (BSA; ≥98% purity), fetal bovine serum (FBS), dimethylsulfoxide (DMSO; ≥99.9% purity) and all FAs (PA, PMA, SA, OA, and U-^{13}C-labeled PA) were purchased from Sigma Aldrich (St. Louis, MO, USA). The following primary antibodies were bought from Cell Signaling Technology (Danvers, MA, USA): p-JNK (catalogue #4671), JNK (#9252), p-p38 MAPK (#9211), p38 MAPK (#9212), p-STAT3 (#9138), STAT3 (#8768), p-ERK1/2 (#9101), ERK1/2 (#4695) and SCD1 (#2438). Primary antibodies for α-tubulin (#ab7291) and ELOVL6 (#ab69857) were purchased from Abcam (Toronto, ON, Canada).

2.2. Cell culture experiments

The SCD1 inhibitor ($SCD1_{inhib}$) was diluted to a stock concentration of 10 μM in DMSO. A final working concentration of 10 nM $SCD1_{inhib}$ was created by further diluting the stock solution in adipocyte culture media, as previously described [16]. 3T3-L1 adipocytes were cultured in 5% CO_2 and 100% humidity at 37°C throughout all experiments. Prior to treatments, cells were maintained in basic media consisting of DMEM supplemented with 5% heat-inactivated FBS and 1% pen–strep. Adipocyte differentiation was conducted as previously described [16]. Briefly, differentiation was induced 2 days postconfluence (*i.e.*, day 0) using an established differentiation cocktail that consisted of IBMX (0.5 mM), Dex (1 μM) and human insulin (5 μg/ml) in basic media. Two days later (*i.e.*, day 2), medium was replaced with maintenance media which consisted of basic media supplemented with human insulin at 5 μg/ml. On day 4, FBS was removed from the media, and the remaining duration of the experiments was conducted in serum-free conditions. During each media change, cells were also treated with either 10 nM $SCD1_{inhib}$ or an equivalent volume of DMSO which served as the control condition.

Stock FA solutions of PA, PMA, SA and OA were made by solubilizing FAs in 100% ethanol (EtOH) [5]. Two percent BSA was prepared directly in serum-free media. FA stock solutions were diluted in the 2% BSA serum-free media to yield an FA:BSA molar ratio of 1:3, as previously described [17,18]. Adipocytes were treated with a final concentration of 250 μM FAs for 48 h, starting on day 5. This dose was selected based on previous work in this adipocyte model [29], as well as work from our laboratory [5]. An equivalent volume of 100% EtOH diluted in 2% BSA serum-free media was used as the control. Cells were still maintained in either 10 nM $SCD1_{inhib}$ or an equivalent volume of DMSO for the duration of FA treatments. FA treatments did not cause cell toxicity, as confirmed using the Promega Cytotoxicity Assay (Madison, WI, USA). All experiments were performed with technical replicates in at least three different passages to ensure that results were not due to passage number.

2.3. Lipid extraction and quantification

Lipid extractions were conducted on day 7 using previously described protocols [16,19,20]. Samples were analyzed using an Agilent Technologies 7890B gas chromatography (GC) system (Agilent Technologies, Mississauga, ON, Canada) and quantified using methods previously outlined [16]. FA data were normalized by cell count for each treatment condition. FA data are reported as μg FA/1×10^6 cells±S.E.M.

2.4. ^{13}PA elongation experiments

To assess if PA elongation to SA is up-regulated during SCD1 inhibition, control cells (D+^{13}PA) and SCD1-inhibited cells (I+^{13}PA) were treated with 250 μM of a U-^{13}C-labeled PA (^{13}PA) stable isotope tracer (Sigma Aldrich, St. Louis, MO, USA). Lipids were extracted from adipocytes using a modified Folch method [21] by the addition of 3 mL of chloroform-methanol with 10 mg of docosatrienoic acid ethyl ester (Nu Chek Prep, Elysian, MN, USA) as an internal standard and 50 mg/mL butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MO, USA) as an antioxidant. Following a 1 min vortex, 0.5 mL of 0.3 M sodium phosphate buffer was added, inverted twice and centrifuged for 5 min at 3000 rpm. The lower organic, lipid-containing phase was pipetted off and transferred to a new glass test tube for transesterification to FA methyl esters as described previously [22].

FA methyl esters were analyzed on a Varian 3900 GC (Bruker, Billerica, MA, USA) for the purpose of quantifying total SA, including the total concentration of co-eluted ^{13}C-labeled SA and nonlabeled SA. The GC settings were as previously described [23]. Peaks were identified by retention times through comparison to an external mixed standard sample (GLC-462, Nu Chek Prep Inc., Elysian, MN, USA).

Relative contributions of ^{13}C-labeled SA to nonlabeled SA were also analyzed, and based on concentrations determined from GC analysis, the concentration of the ^{13}C-labeled SA (M+16) was determined. A Varian 3800 gas chromatograph coupled to a Varian 4000 mass spectrometer with a quadrupole ion trap was utilized (Bruker, Billerica, MA, USA) [24]. The instrument was set for positive chemical ionization with isobutane as the ionizing gas and the reagent ion $C_4H_9^+$ (m/z 57) were mass selected to ionize the FA methyl esters [25]. The GC and mass spectrometer settings were as previously described [24].

2.5. Immunoassays

Media were extracted from adipocytes at day 7 and centrifuged at 1200 rpm for 5 min to pellet any cell debris and stored at −80°C until analyzed. Samples were assessed for the presence of MCP-1, IL-6 and CCL5 using analyte-specific ProcartaPlex immunoassay kits that were purchased from eBioscience (San Diego, CA, USA) and analyzed on a multiplex system with xMAP technology (BioRad Laboratories, Mississauga, ON, Canada).

2.6. RNA and protein extraction

Total RNA and protein were extracted from adipocytes at day 7 using previously described protocols [18]. The Qiagen RNeasy Mini Kit (Qiagen, Mississauga, ON, Canada) was used to extract RNA. A nanodrop and a detergent-compatible protein assay (BioRad Laboratories, Mississauga, ON, Canada) were used to quantify RNA and protein, respectively. Samples were stored at −80°C prior to all analyses.

2.7. Real-time RT-PCR

Single stranded cDNA was synthesized from 1 μg total RNA using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Burlington, ON, Canada). Real-time RT-PCR was conducted as described previously using a BioRad CFX96 RT-PCR detection system and SSo FAST EvaGreen Supermix (BioRad Laboratories, Mississauga, ON, Canada) [16]. Primers for *Scd1*, *Elovl6*, *Mcp-1*, *Il-6* and *Ccl5* were designed using the online Roche Universal Probe Library and Assay Design Center, and *Rplp0* was used as a housekeeping gene.

2.8. Western blot analyses

Western blot analyses were conducted as previously described [16]. Specific primary antibody dilutions were as follows: p-JNK – 1:500; JNK, p-p38 MAPK, p38 MAPK, p-STAT3, STAT3, p-ERK1/2, ERK1/2, SCD1 – 1:1000, α-tubulin – 1:5000, and ELOVL6 – 1:8000. Relative band intensities were quantified using Alpha Innotech Software (San Leandro, CA, USA), and α-tubulin was used as the internal control.

2.9. Statistical analyses

FA and cytokine data were assessed using a one-way ANOVA and Fisher's Least Significant Difference post-hoc analysis. Significant differences in gene expression and protein content between treatment and control conditions were assessed using two-tailed Student's *t* tests. A P-value<0.05 was considered statistically significant. All data are presented as mean±S.E.M.

3. Results

3.1. SCD1 mediates the effects of SFAs and MUFAs on adipocyte FA metabolism

We first examined how total lipid content was influenced by FAs in adipocytes treated with or without $SCD1_{inhib}$. Adipocytes treated with only the inhibitor (*i.e.*, I+EtOH) showed a small but insignificant reduction in total lipid content compared to the control condition (*i.e.*, D+EtOH) (Table 1). Treating control adipocytes with FAs increased total lipid content, and similar increases were also seen in SCD1-inhibited adipocytes treated with FAs (*i.e.*, I+PA, I+PMA, I+SA or I+OA; Table 1). Interestingly, adipocytes treated with I+SA had a significantly higher total lipid content compared to adipocytes treated with I+PA, I+PMA or I+OA.

As expected, treating control adipocytes with individual FAs led to a corresponding increase in their cellular abundance. Specifically, the D+PA, D+PMA, D+SA and D+OA treatments caused significant increases in PA by 2.4-fold, PMA by 2.1-fold, SA by 3.6-fold and OA by 5.3-fold, respectively (Table 1). Similarly, FA treatments in SCD1-inhibited adipocytes also caused substantial increases in PA by 3.1-fold, PMA by 7.2-fold, SA by 6.1-fold and OA by 5.8-fold, respectively (Table 1).

Treating control adipocytes with SFAs (*i.e.*, D+PA or D+SA) increased the abundance of their corresponding desaturated MUFAs, that is, ~2.0-fold increase in PMA (P<0.001) and ~4.6-fold increase in OA (P<0.001). Treating SCD1-inhibited adipocytes with SFAs (*i.e.*, I+PA and I+SA) also increased the content of their corresponding desaturated MUFAs, albeit to a lesser extent (Table 1). The residual conversion of SFAs to MUFAs in SCD1-inhibited adipocytes treated with PA or SA did not stem from changes in *Scd1* gene expression or SCD1 protein content (data not shown). Rather, this conversion most likely stemmed from residual SCD1 activity when using this dose of inhibitor [26]. Furthermore, *Scd2* gene expression was also unaltered by SFA treatments during SCD1 inhibition (data not shown).

Interestingly, treating SCD1-inhibited adipocytes with PA caused a notable and statistically significant increase in cellular SA content compared to the I+EtOH condition (1.5-fold increase, P=0.04; Table 1). We previously reported that the estimated activity of ELOVL6 (*i.e.*, estimated using the product-to-substrate ratio of FAs) was increased in SCD1-inhibited adipocytes [16]; therefore, we repeated this experiment using U-^{13}C-labelled PA. We observed a 5.7-fold increase in labelled SA levels in adipocytes treated with I+^{13}PA relative to the I+EtOH treatment (P<0.001, Fig. 1). *Elovl6* gene expression was significantly up-regulated 1.2-fold in cells treated with I+PA relative to I+EtOH (P=0.03), which is notable given that *Elovl6* expression was already up-regulated 2.4-fold with I+EtOH treatment relative to D+EtOH (P<0.001). ELOVL6 protein content was unaltered by I+PA treatment in adipocytes.

Treating adipocytes with MUFAs did not influence adipocyte FA profiles to the same extent as SFAs. Specifically, the D+PMA treatment did not significantly alter the levels of PA, SA or OA, and the D+OA treatment did not alter PMA or SA content. However, there was a slight 1.2-fold increase in PA content in cells treated with D+OA. Similar results were seen with MUFA treatments in SCD1-inhibited adipocytes, where the I+PMA treatment did not alter PA, SA or OA content (Table 1), and the I+OA treatment did not alter PMA or SA content but did cause a slight 1.2-fold increase in PA levels. The D-PMA treatment

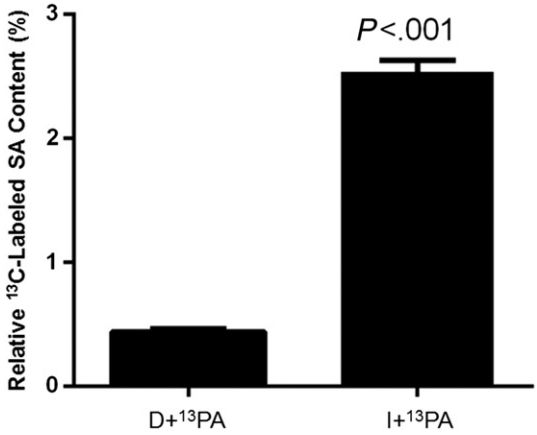

Fig. 1. Relative ^{13}SA content after U-^{13}C-labeled PA treatment. Control and SCD1-inhibited adipocytes were treated with 250 μM U-^{13}C-labeled PA for 48 h. Statistical significance between the conditions was determined used a two-tailed Student's *t* test. D, dimethylsulfoxide; I, SCD1 inhibitor; ^{13}PA, U-^{13}C-labeled palmitate; ^{13}SA, ^{13}C-labeled stearate.

also caused a 1.6-fold increase in vaccenic acid (VA) levels (P<0.001) (data not shown). Moreover, I+PMA increased VA levels significantly more than D+PMA (3.7-fold; P<0.001). These results reflect the increase in ELOVL6 activity seen in SCD1-inhibited adipocytes. However, I+PMA did not appear to influence *Elovl6* gene expression or protein content (data not shown). Treating SCD1-inhibited adipocytes with either MUFA decreased *Scd1* gene expression (1.5-fold decrease with I+PMA (P=0.04) and 1.6-fold decrease with I+OA (P=0.002)). *Scd2* expression was slightly reduced by I+PMA treatment (1.2-fold, P=0.002) but not with I+OA. Protein content of SCD1 and ELOVL6 was unaltered by MUFAs in SCD1-inhibited adipocytes.

3.2. SFA and MUFA treatments do not influence cellular stress in SCD1-inhibited adipocytes

While SFAs such as PA and SA have been reported to activate markers of cellular stress [6,27,28], it is unknown if their effects would be amplified in SCD1-inhibited adipocytes. Furthermore, the effects of MUFAs on cellular stress in SCD1-inhibited adipocytes are unknown. FA treatments in control cells (*i.e.*, D+PA, D+PMA, D+SA and D+OA) did not alter levels of phosphorylated JNK, STAT3, ERK1/2 or p38 MAPK (Figs. 2–3). Treating adipocytes with either I+PA or I+PMA did not

Table 1
Total FA content in adipocytes.

Adipocyte treatment condition	FA (measured by GC)					
	PA	PMA	SA	OA	Other FAs	Total lipid
D+EtOH	22.11±1.21	41.76±2.46	3.94±0.20	13.72±0.57	27.81±1.48	109.33±5.59
I+EtOH	29.78±1.39	15.27±1.15	10.73±0.21	20.46±0.71	25.50±0.95	101.74±4.09
P-value	<**0.0001**	<**0.0001**	**0.0008**	**0.0165**	**0.1000**	**0.2409**
D+PA	52.95±1.78 *	81.6±1.83 *	5.63±0.13	19.74±0.48	35.59±0.97 *	195.52±4.55 *
I+PA	91.72±2.67 **	26.34±1.75 **	15.60±0.51 **	20.76±1.03	29.11±0.72 **	183.52±5.78 **
P-value	<**0.0001**	<**0.0001**	**0.0005**	**0.7957**	**0.0014**	**0.1906**
D+PMA	25.48±1.1	88.6±2.81 *	4.55±0.18	12.4±0.5	29.98±1.07	161.01±5.56 *
I+PMA	31.73±1.36	109.96±4.05 **	11.67±0.37	13.89±0.67	36.03±1.05 **	203.30±6.78 **
P-value	**0.0164**	<**0.0001**	0.0117	0.7026	0.0027	<0.0001
D+SA	31.65±1.33 *	47.27±3.72	14.05±1.79 *	63.36±5.04 *	34.82±1.6 *	191.15±7.38 *
I+SA	55.06±2.42 **	16.42±2.01	65.34±6.51 **	94.65±1.52 **	36.88±1.32 **	268.35±7.08 **
P-value	<**0.0001**	<**0.0001**	<**0.0001**	<**0.0001**	**0.3116**	<**0.0001**
D+OA	26.79±1.25 *	37.14±2.16	3.98±0.29	73.32±6.04 *	27.85±1.41	169.07±7.44 *
I+OA	34.62±1.66 **	10.45±1.17	10.28±0.17	118.42±5.44 **	24.75±0.82	198.52±3.96 **
P-value	<**0.0001**	<**0.0001**	**0.0296**	<**0.0001**	**0.1298**	**0.0022**

Differentiated adipocytes (with or without $SCD1_{inhib}$) were treated with 250 μM PA, PMA, SA or OA for 48 h. One-way ANOVA and Fisher's Least Significant Difference post-hoc test were used to assess for statistically significant differences between conditions. Presented *P*-values correspond to the pairwise comparison of D and I conditions for each FA treatment. "Other FAs" corresponds to the sum of all other FAs measured by GC, excluding PA, PMA, SA and OA.

* Significant difference compared to the D+EtOH control (P<0.05).

** Significant difference compared to the I+EtOH condition (P<0.05).

influence JNK, p38 MAPK, STAT3 or ERK1/2 activity (Fig. 2). We also found no evidence that these stress kinases were altered in adipocytes treated with I+SA and I+OA, although there did appear to be a slight, albeit statistically insignificant, increase in p-JNK and p-p38 MAPK with I+SA (Fig. 3).

3.3. SCD1 mediates SFA and MUFA regulation of cytokine expression and secretion

FAs are known to regulate adipocyte inflammatory signalling pathways [29], and previous work has also shown that SCD1 can mediate inflammatory gene expression in 3T3-L1 adipocytes [11]. We therefore examined if the effects of SFAs and MUFAs on gene expression and protein secretion of common inflammatory markers were exacerbated in SCD1-inhibited adipocytes.

D+PA and D+PMA treatments did not alter *Ccl5* gene expression; however, treating adipocytes with SA and OA up-regulated *Ccl5* gene expression: D+SA – 2.1-fold (P=0.003) and D+OA – 1.5-fold (P<0.001). Conversely, *Mcp-1* gene expression was slightly reduced by D+PA (1.2-fold; P=0.04), but significantly increased by D+SA (1.4-fold; P=0.01). MUFA treatments did not alter *Mcp-1* gene expression in control adipocytes. *Il-6* expression was under the detection limits of qRT-PCR. None of the SFA treatments affected the secretion of CCL5, MCP-1 or IL-6 in control adipocytes (Fig. 4).

In SCD1-inhibited adipocytes, gene expression of *Ccl5* and *Mcp-1* was up-regulated 1.9-fold (P=0.005) and 1.4-fold (P=0.006) with I+EtOH treatment. Upon examining cytokine secretion, CCL5, but not IL-6 or MCP-1, was increased 2.1-fold (P<0.001) with I+EtOH treatment compared to D+EtOH (Fig. 4). While SCD1-inhibition alone did not have a major impact on inflammatory cytokine expression or secretion, treating SCD1-inhibited adipocytes with SA had more profound effects. Specifically, the I+SA treatment caused a significant 3.2-fold (P=0.04) increase in *Mcp-1* and a 5.3-fold (P=0.001) increase in *Ccl5* expression compared to I+EtOH. *Il-6* expression was under the detection limits of qRT-PCR. I+SA treatment also caused a significant 17.8-fold (P<0.001) and 4.0-fold (P<0.001) increase in IL-6 and MCP-1 protein secretion compared to the I+EtOH condition (Fig. 4); however, I+SA did not alter CCL5 secretion.

Of the other FA treatments in SCD1-inhibited adipocytes, I+PA and I+OA significantly increased *Mcp-1* gene expression 1.2-fold (P=0.01) and 1.4-fold (P=0.03), respectively; however, no other FA treatments increased adipocyte MCP-1 secretion. *Ccl5* gene expression was up-regulated in cells treated with I+PA (2.7-fold; P=0.02), unaltered by I+PMA, and decreased with I+OA (1.4-fold; P=0.006) (data not

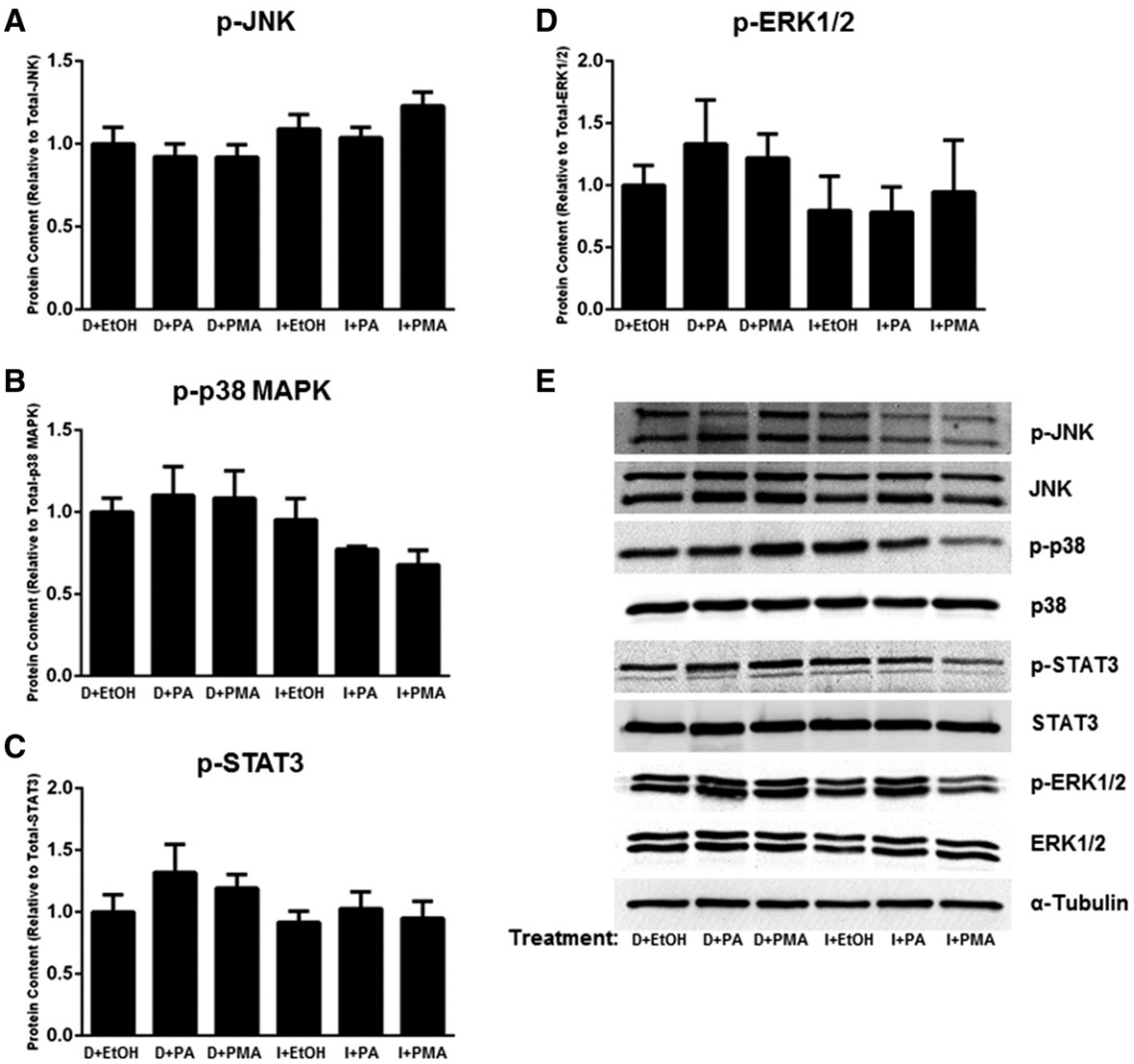

Fig. 2. Markers of cellular stress in adipocytes treated with PA and PMA. Differentiated adipocytes (with or without $SCD1_{inhib}$) were treated with either 250 μM PA or PMA for 48 h. Western blotting was used to assess the protein content of (A) p-JNK, (B) p-p38 MAPK, (C) p-STAT3 and (D) p-ERK1/2. Phosphorylated proteins are expressed relative to their total protein counterpart. Representative blots are shown in (E).

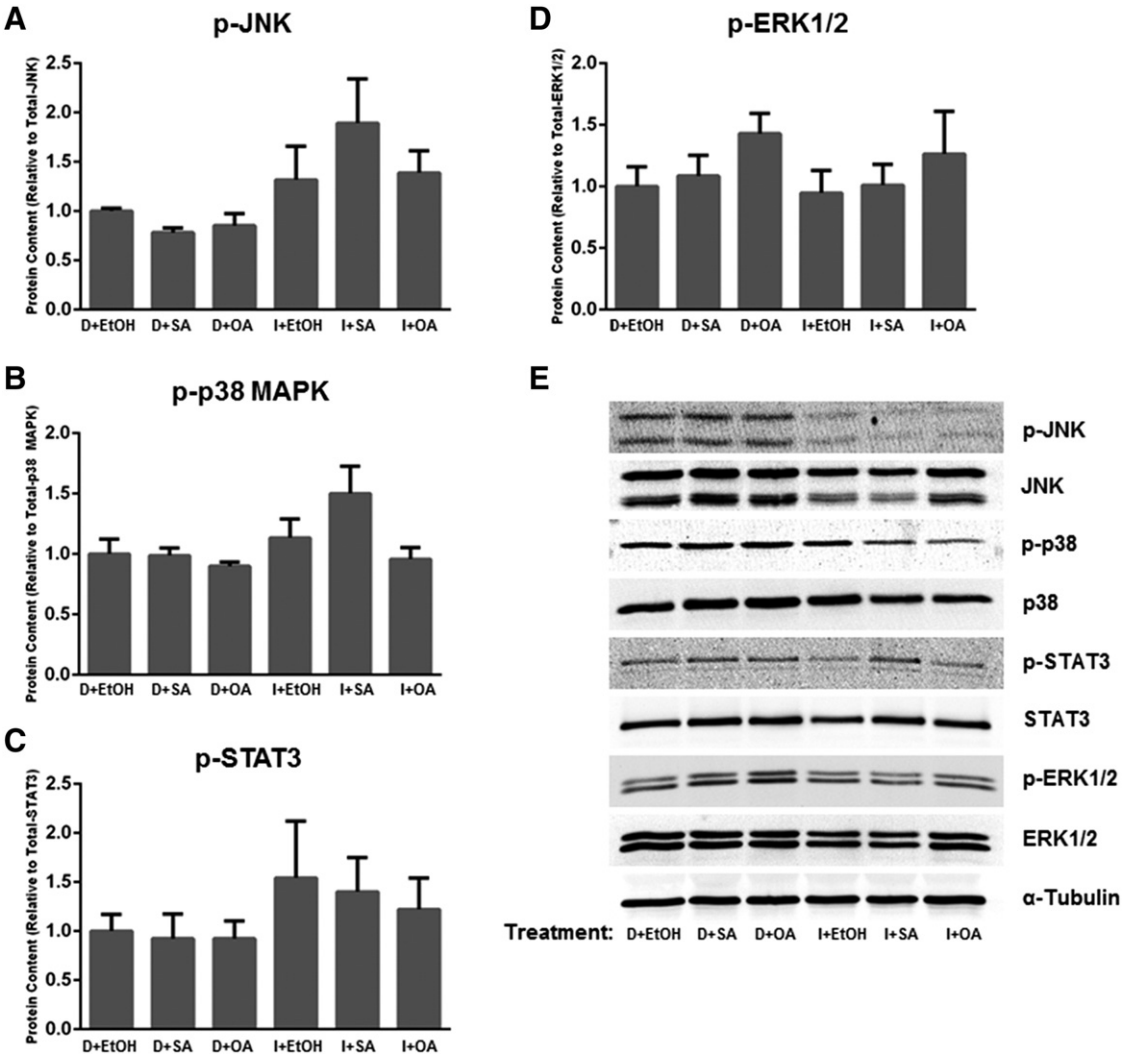

Fig. 3. Markers of cellular stress in adipocytes treated with SA and OA. Differentiated adipocytes (with or without $SCD1_{inhib}$) were treated with either 250 μM SA or OA for 48 h. Western blotting was used to assess the protein content of (A) p-JNK, (B) p-p38 MAPK, (C) p-STAT3 and (D) p-ERK1/2. Phosphorylated proteins are expressed relative to their total protein counterpart. Representative blots are shown in (E).

shown). In contrast, CCL5 secretion appeared to be lower for I+PA compared to I+EtOH, but increased for I+PMA and I+OA (Fig. 4).

4. Discussion

FAs are capable of influencing a myriad of biological processes in AT including inflammatory, metabolic, and insulin signalling pathways [30,31]. Furthermore, previous work has shown that distinct FAs have different effects on the transcriptional profile of adipocytes [5]. Given that the most abundant FAs in AT are linked to SCD1 as either enzyme substrates (*i.e.*, PA and SA) or products (*i.e.*, PMA and OA) [12–15], we sought to examine if a change in SCD1 activity affected FA bioactivity. Specifically, our goal was to study if the influence of FAs on adipocyte inflammatory status, cellular stress and FA profiles is altered when SCD1 activity is compromised.

As expected, FA treatments caused a significant increase in the abundance of the corresponding FAs within adipocytes, regardless of SCD1 inhibition. FAs taken up by adipocytes are normally incorporated into TAG molecules for storage; however, SCD1 inhibition is known to decrease total TAG content both at the tissue level [15,32] and adipocytes [16]. Therefore, despite a compromise in TAG production in SCD1-inhibited adipocytes, it appears as though these cells are still able to take up FAs. This suggests that the FAs are being incorporated into other adipocyte lipid fractions, such as free fatty acids (FFAs), diacylglycerols and cholesteryl esters. This is supported by previous work from our group showing that FFA, diacylglyerols and cholesteryl ester levels are increased in SCD1-inhibited adipocytes [26].

Treating SCD1-inhibited adipocytes with PA (*i.e.*, I+PA) caused an increase in cellular SA levels, which prompted us to examine elongation using a stable isotope tracer. We demonstrated that when SCD1 activity is inhibited, adipocyte ELOVL6 activity is increased. While previous work from our laboratory showed that *Elovl6* gene expression was up-regulated in SCD1-inhibited 3T3-L1 adipocytes [16], this is the first time that this has been demonstrated using a stable isotope tracer. We initially surmised that this increase in ELOVL6 activity may occur in order to mitigate the cellular accumulation of PA, which is purported to promote cellular stress and inflammation [16,33]; however, the results of our analysis of stress markers and cytokine secretion did not support this hypothesis.

Certain stress stimuli (*e.g.*, apoptosis, cytokines and ligands for toll-like receptors) can be increased by FAs and, in turn, can induce intracellular signalling pathways including the jun N-terminal kinase (JNK) system, extracellular signal-regulated kinases (ERK1/2), p38 mitogen-activated protein kinase (MAPK), as well as signal transducer

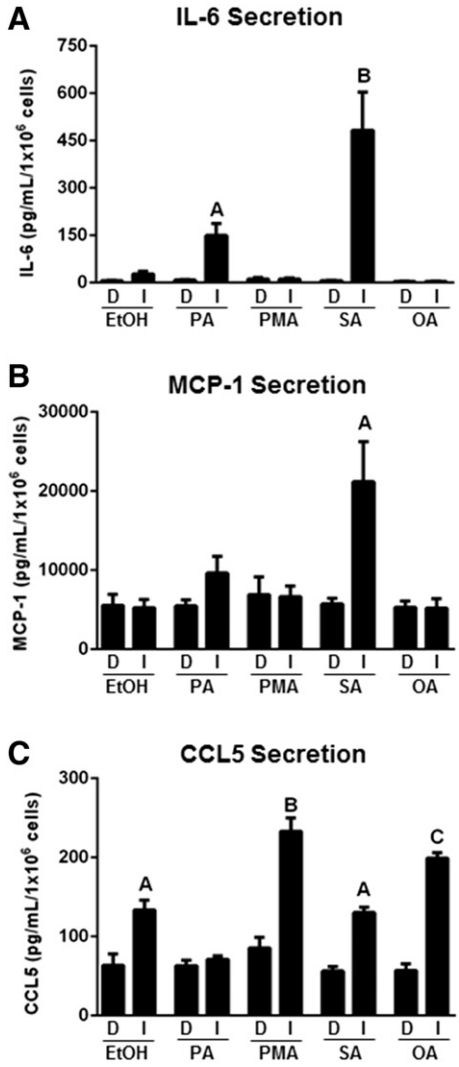

Fig. 4. Cytokines secreted from adipocytes treated with FAs. Differentiated adipocytes (with or without $SCD1_{inhib}$) were treated with either 250 μM PA, PMA, SA or OA for 48 h. Culture media was assessed for secreted (A) IL-6, (B) MCP-1 and (C) CCL5. One-way ANOVA and Fisher's Least Significant Difference post-hoc test was used to assess statistical differences between conditions. Data bars not sharing an identical letter are statistically different ($P<0.05$). Secreted IL-6 from the I+PA condition was borderline significantly different from the control condition ($P=0.06$). IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; CCL5, chemokine (C-C motif) ligand 5.

and activator of transcription 3 (STAT3) [34–38]. However, we found no evidence that FAs altered the activation of JNK, ERK1/2, p38 MAPK or STAT3 in SCD1-inhibited adipocytes compared to controls. The lack of cellular stress in SCD1-inhibited adipocytes treated with SFAs was unexpected, since SFAs have previously been reported to induce the aforementioned signalling cascades [6,27,28]. For instance, Guo *et al.* showed that ERK1/2 and JNK were activated in 3T3-L1 preadipocytes treated with 250 μM PA but not OA [6]; however, these authors conducted their experiments in undifferentiated preadipocytes, while we used differentiated adipocytes. The use of undifferentiated preadipocytes by Guo *et al.* is a notable difference with our work because it was previously postulated that preadipocytes may control p-ERK levels in order for adipogenesis to occur [36]. Since our studies were conducted in differentiated adipocytes, this may explain why we did not see a change in p-ERK levels. Moreover, Koeberle *et al.* also showed that inhibiting SCD1 in murine NIH-3T3 fibroblasts did not influence ERK1/2 or JNK activation [27].

FA doses between 50 μM and 500 μM are considered well within the physiologically relevant range for both human and rodent models [29,39,40]. Our moderate FA dose of 250 μM may provide one possible explanation for the discrepancies between our results and those of previous studies. For instance, work by Yin *et al.* showed that PA treatment in 3T3-L1 adipocytes significantly increased p-JNK protein levels [41]. However, Yin *et al.* observed these effects using a PA dose of 500 μM; whereas in our study, 250 μM PA did not alter p-JNK levels. This suggests that a higher dose of PA may be necessary in order to induce JNK signalling in adipocytes. Differences in FA treatments may also explain discrepancies between our findings regarding p38 MAPK and those reported by Koeberle *et al.*[27]. While we observed no effect with 250 μM FA treatments on p38 MAPK activation in SCD1-inhibited adipocytes, Koeberle *et al.* reported that higher doses (*i.e.*, 400 μM) of SFAs activated p38 MAPK in NIH-3T3 cells [27]. Finally, the fact that we did not see a change in p-STAT3 levels in SCD1-inhibited adipocytes was not entirely surprising since STAT3 is downstream of p38 MAPK, JNK or ERK1/2 [42,43].

There is currently a lack of consensus regarding SCD1's influence on inflammatory signalling in AT or adipocytes [44]. While some studies have reported that SCD1 inhibition promotes AT inflammation [44], others have shown that reduced SCD1 can prevent inflammation [10]. The inconsistent findings concerning the relationship between SCD1 and inflammation stem largely from the use of different experimental model systems (*e.g.*, adipocyte cell lines, wild type rodent AT or AT isolated from *Scd1*—/— mice). The current study used the well-established 3T3-L1 adipocyte cell line to explore the role of SCD1 as a mediator of FA-regulation of inflammation. This experimental approach avoids confounders, such as potential compensatory effects in global knock-out models and the role of other cell-types (*e.g.*, macrophages, T-cells, endothelial cells) in AT. However, we acknowledge that we cannot directly translate our results to *in vivo* models without further investigations.

Although none of the FA treatments altered the secretion of common markers of inflammation (*i.e.*, IL-6, MCP-1 and CCL5) in control adipocytes, we found that the combination of SCD1 inhibition and SFA treatments caused a significant increase in the secretion of these inflammatory markers. Therefore, while normal adipocytes may be able to effectively "handle" FAs without triggering the production of inflammatory signals, this appears to be compromised when SCD1 activity is inhibited. Of the four FAs used in the present investigation, SA evoked the greatest changes in inflammatory gene expression and protein secretion. Treating control adipocytes with SA increased the expression of *Ccl5* and *Mcp-1*; however, these effects were exacerbated in SCD1-inhibited adipocytes. Further, this was evidenced at the level of both gene expression and cytokine secretion. While previous investigations have demonstrated that PA promotes MCP-1 and IL-6 secretion [45] and gene expression [46,47] in 3T3-L1 adipocytes, these prior studies did not examine the effects of SA. Schaeffler *et al.* conducted one of the few studies examining the influence of SA on cytokine secretion from adipocytes, and they found that 100 μM SA induced MCP-1 secretion but to a lesser extent than 100 μM PA [48]. While PA and SA are both saturated FAs, we have previously shown that they trigger distinct transcriptional responses in adipocytes [5]. Our results suggest that when SCD1 activity is compromised in adipocytes, SA appears to have greater proinflammatory effects compared to PA. This may stem from an overload of SA, since the combination of SA treatment and increased production via ELOVL6 contribute to SA levels that are ~17-fold greater than those of control adipocytes.

We acknowledge the limitation that we have only targeted SCD1 in our analyses despite the fact that adipocytes also express SCD2. However, SCD1 is the dominant isoform in AT [8,49]. In addition, future work should continue to examine the influence of individual FAs on the paracrine dialogue (*i.e.*, crosstalk) that exists between the

different cell-types residing in AT. This is relevant given our observations that FAs altered the inflammatory protein secretion from SCD1-inhibited adipocytes, which may affect the activity of AT immune cells (*e.g.*, macrophages). Finally, future work should consider examining various FA concentrations in order to assess if cellular stress responses are exacerbated with higher FA doses.

5. Conclusion

The present study has provided novel information regarding how SCD1 mediates the effects of SFA and MUFA treatments in adipocytes. FAs had different effects on adipocyte lipid profiles, as well as inflammatory cytokine gene expression and secretion, when SCD1 activity was compromised. Specifically, we showed that the influence of FAs on inflammatory markers was exacerbated when adipocyte SCD1 activity was inhibited. In addition, SA appeared to be more proinflammatory than PA in SCD1-inhibited adipocytes, as evidenced by larger increases in inflammatory cytokine secretion and expression. We also demonstrated that PA elongation to SA was up-regulated in adipocytes when SCD1 activity was compromised. These results not only highlight the differential effects of individual FAs but advance our knowledge regarding how SCD1 can mediate the bioactivity of FAs in adipocytes.

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