De Novo Lipogenesis and Stearoyl-CoA Desaturase Are Coordinately Regulated in the Human Adipocyte and Protect against Palmitate-induced Cell Injury* [S]

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De novo lipogenesis (DNL) is paradoxically up-regulated by its end product, saturated fatty acids (SAFAs). We tested the hypothesis that SAFA-induced up-regulation of DNL reflects coordinate up-regulation of elongation and desaturation pathways for disposal of SAFAs and production of monounsatuated fatty acids to protect cells from SAFA toxicity. Human preadipocytes were differentiated in vitro for 14 days with [U-13C]palmitate (0–200 μM) to distinguish exogenous fatty acids from those synthesized by DNL. Exogenous palmitate up-regulated DNL (p < 0.001) concomitantly with SCD and elongation (each p < 0.001). Adipocytes from some donors were intolerant to high palmitate concentrations (400 μM). Palmitate-intolerant cells showed lower TG accumulation. They had lower expression of SCD mRNA and less monounsaturated fatty acids in TG, emphasizing the importance of desaturation for dealing with exogenous SAFAs. There was greater [U-13C]palmitate incorporation in phospholipids. SCD knockdown with small interfering RNA controls. There was preferential channeling of DNL-derives versus exogenous palmitate into elongation and of DNL-derived versus exogenous stearate into desaturation. DNL may not act primarily to increase fat stores but may serve as a key regulator, in tandem with elongation and desaturation, to maintain cell membrane fluidity and insulin sensitivity within the human adipocyte.

In many cell types, including pancreatic β-cells and endothelial cells, the saturated fatty acid (SAFA) palmitate can have adverse effects on cell function, including endoplasmic reticulum stress and apoptosis (1–4). Palmitate-induced apoptosis can be rescued by the monounsaturated fatty acids (MUFAs) palmitoleate (16:1 n-7) and oleate (18:1 n-9), preventing apoptosis and increasing fatty acid storage as triglyceride (TG) (5, 6). Additionally, the adverse effects of an overabundance of saturated fatty acids have been well documented in humans (7, 8).

De novo lipogenesis (DNL) is the formation of lipids from nonfat precursors such as glucose and produces the SAFAs myristate (14:0, a minor end product) and palmitate (16:0, the main end product). Palmitate and stearate (18:0) are substrates for stearoyl-CoA desaturase (SCD, or Δ-9 desaturase), which acts to convert these SAFAs to MUFAs palmitoleate and oleate, respectively. In the liver, the pathways of DNL and fatty acid desaturation by SCD appear to be coordinately regulated (9). Therefore, it would seem that SCD plays a crucial role in maintaining the intracellular equilibrium of SAFAs and MUFAs. However, the literature surrounding the role of SCD in cell function and disease is conflicting.

Paradoxically, SAFAs have been shown to up-regulate lipogenesis. Early studies in rats showed palmitate to stimulate glucose incorporation into TG fatty acids (10, 11). Several studies of high fat feeding in rats and mice, whether or not specifically high in saturated fat, show induction of DNL at a transcriptional or flux level (12–15). In humans, Warensjö et al. (16) recently reported an increased SCD index (16:1 n-7/16:0) in serum phospholipids (PLs) and cholesteryl esters in subjects fed a high saturated fat diet compared with those fed a rapeseed oil-rich diet, implying an up-regulation of SCD by SAFAs.

We hypothesized that the paradoxical up-regulation of DNL seen in response to high fat feeding or SAFA exposure might represent an integral activation of fatty acid modification (desaturation and elongation) with DNL. Thus, as cells attempt to reduce SAFA accumulation by metabolic transformation of the fatty acids, DNL may necessarily be activated in parallel. DNL together with elongation and desaturation would also provide a source of oleate to ameliorate the adverse effects of palmitate excess.

We have investigated this in human adipocytes as a model primary human cell; there is considerable evidence now for the importance of DNL in adipocytes (17–19). We have investigated the regulation of these pathways and how they may be affected by an exogenous source of palmitate, the end product of DNL. A functional link between DNL and SCD elongation might also be evident in channeling of fatty acids through these pathways, which we have investigated with stable isotope tracer methodology. Our hypothesis makes some critical predictions. One is that cells that fail to up-regulate DNL and desaturation
pathways appropriately will be adversely affected by provision of exogenous palmitate. Another is that the loss of SCD would be accompanied by a down-regulation of DNL, which we have tested with siRNA silencing of SCD. We also investigated the effects of SCD loss-of-function on DNL and adipocyte function, particularly cell membrane fluidity and insulin sensitivity, in the presence and absence of exogenous palmitate.

**EXPERIMENTAL PROCEDURES**

**Adipose Tissue Sample Collection**—The taking of human adipose tissue samples was approved by the Oxfordshire Clinical Research Ethics Committee, and all subjects gave written, informed consent. Subcutaneous adipose tissue biopsies were obtained by needle aspiration using a 12-gauge needle. Tissue donors consisted of 11 males and 11 females, with a median age of 38 years (ranging from 21 to 53 years) and with a median body mass index of 25.8 kg/m² (ranging from 21.0 to 40.1 kg/m²).

**Preadipocyte Isolation and Differentiation**—Adipose tissue biopsies were taken from abdominal subcutaneous adipose tissue of healthy volunteers using a 12-gauge needle and syringe. Preadipocytes were isolated from subcutaneous adipose tissue as described by Hauner et al. (20). Adipose tissue was mechanically minced using scissors, washed twice with Hanks’ buffered salt solution to remove contaminating blood, and then enzymatically digested in 1 mg/ml collagenase (Roche Applied Science) in Hanks’ buffered salt solution to remove any large cells. The digested tissue was centrifuged at 1000 rpm for 5 min at 4 °C, and the supernatant was removed and resuspended in growth medium (Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 Ham’s (v/v, 1:1), 10% fetal calf serum (Invitrogen), 1% human insulin, 17 μM pantothenate, 100 nM human insulin, 1 nM triiodo-L-thyronine, 33 μM biotin, 10 μg/ml transferrin, 1 μM dexamethasone, and 1 ml/liter gentamicin. 3-Isobutyl-1-methylxanthine (250 μM) and troglitazone (2 μM) were added for the first 3 days.

If no exogenous fatty acids are provided during differentiation, the TG present after 14 days has necessarily all arisen from DNL and SCD as the sum of all TG fatty acids produced by Δ-9 desaturation.

**Gas Chromatography Analysis of Triglyceride and Phospholipid Fatty Acids**—Known amounts of internal standards of phosphatidylcholine dipentadecanoyl (15:0) and glyceryl triheptadecanole (17:0) were added to samples prior to extraction. The total lipids were extracted from cells using the method described by Folch et al. (21). TG and PL fractions were separated using solid phase extraction columns as described previously, and fatty acid methyl esters were prepared using methanolic sulfuric acid at 80 °C for 2 h (22).

GC was performed using an Agilent 6890N GC (Agilent Technologies, Stockport, UK) fitted with a 25-m × 0.25-mm capillary column (FFAP-CB), with stationary phase composed of polyethylene glycol nitroterephthalic acid ester chemically bonded to a fused silica column and a flame-ionization detector. The carrier and make-up gasses were helium and nitrogen, respectively, with a column flow of 1 ml/min, and the samples were run using a splitless injection technique. The GC operated at an initial temperature of 50 °C with a series of programmed temperature increments to 240 °C, enabling the resolution of fatty acids from 8:0 to 24:0 within 46 min. Individual fatty acid peaks were identified by reference to known FAMEs. The fatty acid concentrations were calculated relative to the internal standard, and the results were expressed as μg of fatty acid/10^6 cells.

**Use of Stable Isotopes to Trace the Fate of Exogenous Palmitate**—[U-13C]palmitate (CK Gas, Cambridgeshire, UK) was also added during differentiation of preadipocytes to trace the fate of exogenous palmitate with SCD knockdown and to assess possible SCD substrate channeling. Concentrations of 0, 50, 100, and 200 μM [U-13C]palmitate were added to the differentiation medium, and GC-mass spectrometry (19) was used to distinguish exogenous from DNL-derived fatty acids in TG and PL fractions. Fatty acids derived from DNL under these conditions will be 13C-labeled, whereas those arising from exogenous sources will be 13C-labeled.

The GC was equipped with a DB-Wax 30m capillary column (from Agilent; inner diameter, 0.25 mm; film thickness, 0.25 μm). The sample (1.0 μl) was introduced using the splitless injection mode, an injector temperature of 250 °C, and an initial oven temperature of 80 °C for 5 min. The oven temperature was increased by 25 °C/min to 200 °C and held for 10 min and then further increased by 25 °C/min to 230 °C, where it was held for 2 min. Mass-to-charge ratios (m/z) were determined by selected ion monitoring. Dwell time was 50 ms.

**SCD Knockdown**—SCD knockdown was achieved using Lipofectamine 2000 (Invitrogen) as a carrier. Because siRNA transfection of cells induces a transient knockdown of mRNA, siRNA was added to differentiating preadipocytes at day 6 of differentiation, when SCD mRNA would be expressed (data not shown), and again at days 9 and 12 (a total of three times) to maintain knockdown. Two different SCD-specific siRNAs were used to achieve SCD mRNA knockdown, termed SCD siRNA1 and siRNA2 (Stealth siRNA, HSS109499 and HSS109500, respectively; Invitrogen), with controls of Lipofectamine only, scrambled siRNA negative control (Stealth Negative siRNA...
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12935–300 (GC content matched to SCD-specific siRNAs; Invitrogen), and one control with no transfection components. The extent of SCD mRNA knockdown was measured as a percentage compared with the scrambled siRNA.

Gene Expression Measurements—The cells were harvested in 0.5 ml of Tri Reagent (Ambion) and transferred to a 2-ml PhaseLock gel Eppendorf tube (catalog number. 955154045; Eppendorf). 100 μl of chloroform was added, shaken by hand for 15 s, and incubated at room temperature for 2–3 min before centrifugation at 12,000 × g for 15 min at 4 °C. The upper RNA-containing aqueous phase was transferred to a clean tube, and an equal volume of isopropanol was added. The samples were precipitated at −80 °C overnight. RNA was then collected by centrifugation at 12,000 × g for 20 min at 4 °C, isopropanol aspirated off, and then the pellets were washed with 0.5 ml of 70% ethanol. The pellets were once again collected by centrifugation at 7,500 × g for 5 min at 4 °C, ethanol was aspirated off, and the pellets were then air dried at room temperature. Once dry, RNA was resuspended in 10 μl of RNA storage solution (Ambion). Any contaminating genomic DNA was digested using 1 μl of DNase buffer and 0.5 μl of DNase I (DNA-free™; Ambion).

cDNA was synthesized by reverse transcription from 500 ng of RNA using a SuperScript III first strand synthesis system (Invitrogen). A cDNA standard was created from a pool of each sample and diluted 1/50, 1/100, 1/500, and 1/1000 in 10 mM Tris, pH 8.0. All of the samples were diluted 1/100, and mRNA expression was analyzed in triplicate by real time PCR using the Applied Biosystems (Warrington, UK) Assays-on-Demand TaqMan® gene expression assays with TaqMan® Universal PCR Master Mix (Applied Biosystems) and run on either a Rotorgene real time PCR machine (Corbett Research, Sydney, Australia) or an Applied Biosystems 7900HT real time PCR machine (Applied Biosystems) in a final volume of 8 μl (3.6 μl of 1/100 dilution of template, 4 μl of Master Mix, and 0.4 μl of specific Assay-on-Demand). The target genes were as follows: ACACA (acetyl-CoA carboxylase), FASN (fatty acid synthase), ACLY (ATP-citrate lyase), G6PD (glucose 6-phosphate dehydrogenase), SCD (stearoyl-CoA desaturase), ELOVL6 (ELOVL family member 6, elongation of long chain fatty acids), and SREBF1 (SREBP1c), assay identifications Hs00167385_m1, Hs00188012_m1, Hs00153764_m1, Hs00166169_m1, Hs00748952_s1, Hs00225412_m1, and Hs00231674_m1, respectively. mRNA expression was calculated using the Δ–Ct transformation method (Q = E[min Ct – sample Ct]) as previously described (23, 24). All of the target genes were normalized to the geometric mean of three housekeeping genes: PPIA (cyclophilin), PGK1 (phosphoglycerate kinase 1), and S18 (eukaryotic 18 S rRNA), assay identifications Hs99999906_m1, Hs99999904_m1, and Hs99999901_s1, respectively.

Measurements of Cell Membrane Fluidity—Human preadipocytes were grown on glass coverslips and differentiated for 14 days. Before fluorescence recovery after photobleaching (FRAP) analysis, the cells were washed in PBS (room temperature) and then incubated with 0.5 μl/ml 100 mM BODIPY® 500/510 C1, C12 (4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid) (Invitrogen) for 2 min at 37 °C. The cells were washed with PBS, and the coverslip was placed on the confocal microscope for analysis.

The images were acquired using an LSM510 confocal microscope and software (Zeiss) using a 488-nm argon ion laser for excitation and ×63/1.4 NA (numerical aperture) objective with light collected through a 515-nm long pass filter. The imaging frames were acquired at 4 Hz (4 frames/second) with an image depth of 8 bits. Background fluorescence was assessed by measurements taken 15 s prior to bleaching. For each condition, the measurements were made on 15 different cells.

BODIPY molecules are not entirely nonpolar. Because there was some labeling of intracellular structures, the contribution of internally labeled organelles to plasma membrane fluidity measurements was minimized by using a confocal section of only 1 μm for scanning and focusing on the bottom of the coverslip to minimize stray fluorescence. Any small contribution from labeled organelles was then held constant, i.e. in every group measured this was the case. We believe this contributed some noise to the small, but significant differences that we report. The data were analyzed using the Metamorph (version 7.1) software package. Two methods of calculation were used. First, all of the intensities were normalized to the intensity at frame 1, and recovery was calculated as the time taken to half-maximum recovery. Second, intensities were normalized to a value of 1.0 at maximum recovery, and curve fitting was used to assess recovery rates. Exploration of the data in this way showed three distinct exponential periods (0–20, 20–60, and 60–180 s). Values of r² using this approach were from 0.975–1.000. The data are given for exponentials fitted over these periods.

Insulin Sensitivity Measurements—Uptake of 2-deoxy-d-[3H]glucose ([3H]2-DOG) is an established method for assessing the insulin sensitivity of adipocytes (25–27). Uptake of [3H]2-DOG was measured in response to three different insulin concentrations (0, 10, and 100 nM) to establish a dose response. The adipocytes were washed three times in warm PBS and then incubated at 37 °C for 2 h in Krebs Ringer HEPES buffer (119 mM NaCl, 4.75 mM KCl, 5 mM NaHCO3, 1.2 mM MgSO4, 1.18 mM KH2PO4, 2.54 mM CaCl2, and 20 mM HEPES, pH 7.4, plus 0.01% bovine serum albumin) containing 5 mM glucose. After 2 h, the 5 mM glucose Krebs Ringer HEPES buffer was removed, and the adipocytes were incubated for 30 min at 37 °C with 1.8 ml of Krebs Ringer HEPES buffer containing 0, 10, or 100 nM insulin (Invitrogen). After 30 min, 200 μl of 2-DOG mixture (containing 37kBq [3H]2-DOG and 2 mM unlabeled 2-DOG) was added for a further 10 min. The wells were then carefully washed three times with ice-cold PBS and harvested in 1 ml of Triton X-100 lysis buffer (1% Triton X-100, 20 mM Tris, and 150 mM NaCl). The cell lysates were transferred to scintillation vials, and 7 ml of scintillation fluid (OptiPhase Safe; Fisher) were added and then counted on a scintillation counter (LSs 6500 Multi-Purpose Scintillation Counter; Beckman).

All of the measurements of 2-DOG uptake were corrected for FABP4 mRNA expression, a marker of adipocyte differentiation. This was chosen as a denominator because previous studies showed exposure of Ob1771 preadipocytes to palmitate increased differentiation (28, 29). Furthermore, the extent of differentiation of 3T3-L1 preadipocytes has been associated with changes in insulin-stimulated 2-DOG uptake (30).
Statistical Analyses—The differences between control and palmitate-treated cells were statistically analyzed using repeated-mesures analysis of variance (ANOVA). This was also used to assess the effects of exogenous palmitate and insulin concentrations and SCD knockdown in the insulin sensitivity assays. Differences between scrambled siRNA controls and SCD-specific siRNAs were assessed using a Student’s t test (paired). All of the statistical analyses were carried out using SPSS (version 15).

RESULTS

Exogenous Palmitate Up-regulates DNL and SCD as Well as Fatty Acid Elongation—The effects of exogenous palmitate on DNL and fatty acid modification were assessed by differentiating preadipocytes for 14 days with 0, 50, 100, or 200 μM [U-13C]palmitate to allow exogenous palmitate (13C-labeled) to be distinguished from DNL-derived palmitate (unlabeled). Total DNL flux (Fig. 1A) was increased in response to exogenous palmitate (p = 0.001, ANOVA), especially at lower concentrations (110% increase at 50 μM exogenous palmitate). Flux through SCD, calculated as the sum of all TG fatty acids made via SCD action, was also up-regulated by exogenous palmitate, as was flux through fatty acid elongation (Fig. 1A; p < 0.001 for each). There was an increase in cellular TG content in response to exogenous palmitate that reflected increases in both DNL and incorporation of exogenous (13C-labeled) palmitate (Fig. 1B; p < 0.001 for each). Expression of mRNA for DNL-associated genes and SCD were also up-regulated (p < 0.05) (Fig. 1C), with a tendency to an increase in ELOVL6 mRNA (p = 0.13).

High Palmitate Has Adverse Effects on the Adipocytes from a Subpopulation of Donors—Differentiation of preadipocytes from 15 donors with 400 μM palmitate revealed that the adipocytes from some donors showed reduced cell viability, whereas others thrived. Adipocytes that survived the high, 400 μM palmitate were classed as palmitate-tolerant (n = 10), and those that did not were classed as palmitate-intolerant (n = 5). Classification was determined solely by observation of significant cell death, as seen by large areas of cell debris interspersed with occasional, very large lipid droplets, in the presence of 400 μM palmitate (Fig. 2A).

Palmitate tolerance or intolerance appeared to be a characteristic of individual donors. All of the cultures derived from one donor behaved in the same way. Repeated biopsies were obtained from two donors, one of whose adipocytes were tolerant and the other intolerant on the first occasion, and the characteristics were maintained with a second biopsy. Various anthropometric parameters were measured from the adipose tissue donors, such as body mass index, waist-to-hip ratio, age, gender, or percentage body fat. We found no significant differences between the donors of palmitate-tolerant and palmitate-intolerant adipocytes (p > 0.15). The full data are in supplemental Table S1.

Palmitate-intolerant Adipocytes Have Reduced DNL and SCD Compared with Palmitate-tolerant Adipocytes and Higher Saturated Fatty Acid Content in Phospholipids—We investigated what characteristics of the adipocytes would render them tolerant or intolerant to high palmitate concentrations. Palmitate-intolerant cells showed less total TG accumulation (tolerant, 380 ± 78; intolerant, 87 ± 30 nmol/10^6 cells; p = 0.040), supporting the morphological observations. Expression of DGAT2 mRNA (coding for a key enzyme in TG synthesis) was lower by 91.8% (p < 0.001) in the palmitate-intolerant cells.

The DNL-derived fatty acid content of adipocyte TG was less in the palmitate-intolerant compared with the palmitate tolerant cells, and mRNA expression for DNL-associated genes was also significantly lower (Fig. 2, B and C). Concomitant with lower activity of DNL in the palmitate-intolerant adipocytes, flux through SCD (Fig. 2D) and SCD mRNA expression (Fig. 2C) were also lower than in the palmitate-tolerant. Furthermore, the proportion of SAFAs in TG (mol%) was higher in the palmitate-intolerant adipocytes than in the palmitate-tolerant (83.6 ± 5.9 and 64.1 ± 0.9, respectively, p < 0.001).

![Figure 1. Changes in DNL, SCD, and elongation in response to exogenous palmitate. A, Flux through DNL (black), SCD (light gray), and elongation (dark gray) increased in response to exogenous palmitate (50–200 μM). B, TG palmitate (16:0), both DNL-derived (black) and exogenously derived (light gray), increased in response to exogenous palmitate. Units for A and B are nmol/10^6 cells over 14 days. C, expression of SCD mRNA and DNL-associated genes was also up-regulated by exogenous palmitate. The data are represented as the means ± S.E. (n = 15).](image-url)
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The PL SAFA:MUFA ratio was more than 2.5-fold higher in the palmitate-intolerant adipocytes than in the tolerant group \((p = 0.001)\). The SAFA:MUFA ratio was 1.43 in the palmitate-tolerant group, whereas it was 0.3 in the intolerant adipocytes. Furthermore, there was greater incorporation of exogenous palmitate into the PL fatty acids than in the tolerant group \((p = 0.051)\).

Effects of SCD Knockdown on DNL—Because desaturation appeared to be important for cell tolerance of exogenous palmitate, we investigated the effects of SCD knockdown in the absence or the presence of 200 \(\mu\text{M} [\text{U-}^{13}\text{C}]\text{palmitate}\). This was achieved using two different siRNA oligonucleotides, which were compared with a scrambled siRNA control. Both siRNAs produced a consistent, stable knockdown over the 72-h period following transfection. The degrees of knockdown were 66.9 \pm 4.5\% for siRNA1 and 87.7 \pm 1.8\% for siRNA2 \((p < 0.001)\). There was an increase in proportion (mol\%) of the SAFA palmitate \((44.7 \pm 1.2 \text{ versus } 48.1 \pm 1.3 \text{ and } 48.0 \pm 1.6 \text{ for control, siRNA1, and siRNA2, respectively})\) and stearate \((4.3 \pm 1.1 \text{ versus } 11.8 \pm 2.1 \text{ and } 11.5 \pm 1.9 \text{ for control, siRNA1, and siRNA2, respectively})\) in TG fatty acids in response to SCD knockdown \((p < 0.001)\).

DNL gene expression was down-regulated in response to SCD knockdown (Fig. 3, top panel). TG fatty acids were also analyzed in preadipocytes differentiated with either 0 or 200 \(\mu\text{M} [\text{U-}^{13}\text{C}]\text{palmitate}\) and are shown in Fig. 3, bottom panel. There was a tendency for a reduction in DNL-derived palmitate with SCD knockdown.

SCD Knockdown Reduces Cell Membrane Fluidity and Impairs Insulin Sensitivity of the Adipocyte—Product:precursor ratios of PL fatty acids reflecting SCD action are shown in Fig. 4. Product:precursor ratios were significantly reduced with SCD knockdown. This meant that SCD knockdown caused a significant \((p = 0.042)\) increase in PL SAFA:MUFA ratios in cells with no added palmitate \((0.89 \pm 0.05 \text{ versus } 1.19 \pm 0.10 \text{ and } 1.14 \pm 0.07 \text{ for control, siRNA1, and siRNA2, respectively})\) and in 200 \(\mu\text{M} \text{palmitate-treated cells (1.31} \pm 0.16 \text{ versus } 1.73 \pm 0.24 \text{ and } 1.64 \pm 0.22 \text{ for control, siRNA1, and siRNA2, respectively})\). There was a borderline significant difference in SAFA:MUFA between the 0 and 200 \(\mu\text{M} \text{palmitate-treated cells (p = 0.051). More detail on phospholipid fatty acid composition is given in supplemental Table S2.}

Analysis of cell membrane fluidity, using FRAP, also revealed that the loss of SCD led to a decreased fluidity (Fig. 5, A and B). Half-times to recovery are shown on Fig. 5B. As described under “Experimental Procedures,” we also fitted exponentials to the recovery curves. The effects were mainly seen in the 0–20- and 20–60-s periods. The exponential coefficients were significantly lower (recovery slower) for siRNA treatment \((0.22 \pm 0.04 \text{ for control, siRNA1, and siRNA2, respectively})\) and in 200 \(\mu\text{M} \text{palmitate-treated cells (0.17} \pm 0.02 \text{ and 0.044 for 0–20 and 20–60 s, respectively})\). For illustration, the size of the effect was quantified in terms of diffusion coefficient \((\mu\text{m}^2/\text{cm})\). For one individual the values were 1.78 \(\pm 0.24 \text{ and } 1.24 \pm 0.15 \text{ for siRNA1 and siRNA2, respectively (14–15 cells for each treatment, p < 0.001 and p = 0.026 by unpaired t test).}

Insulin sensitivity was determined from uptake of radiolabeled 2-DOG in response to insulin. The adipocytes differenti-
ated with 50 and 200 μM palmitate showed reduced insulin sensitivity compared with those differentiated without any exogenous palmitate (p < 0.021) (data not shown). Therefore, the effects of preventing SAFA conversion to MUFA via SCD knockdown were investigated. SCD siRNA-treated cells showed lower insulin-stimulated uptake of 2-DOG (Fig. 5C).

Channeling of Fatty Acids toward Elongation and Desaturation—Given the coordinate regulation of DNL, SCD, and elongation observed above, we next investigated whether fatty acids derived from DNL were preferentially channeled into further modification. DNL-derived palmitate was preferentially channeled toward elongation over exogenous palmitate (p = 0.022) (Fig. 6A). Furthermore, stearate made from elongation of DNL-derived palmitate was preferentially channeled toward SCD-mediated desaturation (p < 0.001) (Fig. 6B). There was no channeling of either DNL-derived or exogenous palmitate toward SCD (p = 0.361).

These distinctions were maintained in the presence of SCD knockdown with siRNA. There was again preferential channeling of DNL-derived stearic acid toward SCD (p = 0.048, repeated measures ANOVA). Furthermore, SCD knockdown also had no effect on the preferential elongation of DNL-derived palmitate. As before, the action of SCD on palmitic acid was not dependent upon the source of this fatty acid, whether from DNL or exogenously derived (p = 0.86).

DISCUSSION

In agreement with previous findings of DNL up-regulation by SAFAs (10–12), we found that exogenous palmitate also up-regulated DNL in human adipocytes. We showed that up-regulation of DNL was accompanied by increased elongation and desaturation and indeed that these pathways are function-
ally coupled, with channeling of palmitic acid derived from DNL through elongation and into oleic acid production. We also showed that down-regulation of SCD with siRNA reduced DNL and had adverse effects on the cell. We identified populations of adipocytes that were unable to tolerate high concentrations of exogenous palmitate; these populations were cells that failed to up-regulate DNL and desaturation appropriately. It would seem counter-intuitive that provision of the end product of DNL, palmitate, should up-regulate this process. However, the finding that SCD and ELOVL6 activities were also increased by palmitate provides a plausible explanation. Because palmitate is a substrate for these enzymes, it appears that the cell up-regulates enzymes capable of modifying this SAFA and converting it to oleic acid. These findings mirror the in vivo finding that DNL and SCD are coordinately up-regulated in response to short term high carbohydrate feeding in humans (9).

Furthermore, there was specific channeling of DNL-derived fatty acids over exogenous fatty acids toward elongation and desaturation. Substrate specificity of SCD is a phenomenon that has previously been described in hepatocytes (31). Specifically, DNL-derived palmitate was channeled toward elonga-
tion to stearate, which in turn was channeled toward SCD to produce oleate. The potential importance of oleate within the cell is 2-fold. First, endogenously derived oleate may be crucial for TG synthesis because DGAT2 (the enzyme that catalyzes the terminal step in TG formation) and SCD are co-localized on the endoplasmic reticulum (32, 33). Palmitate up-regulation of \textit{DGAT2} mRNA may occur as a knock-on effect of SCD up-regulation, enhancing TG formation. Second, oleate is one of the most abundant fatty acids in the cell membrane, and DNL may provide a source of this fatty acid for PL synthesis. Therefore, it could be that the shunting of endogenously produced palmitate toward oleate synthesis is a physiological mechanism for the adipocyte to optimize TG storage and maintain cell membrane fluidity.

The importance of SCD to cell function in the presence of SAFAs was also highlighted when preadipocytes were differentiated with high concentrations of palmitate. Unexpectedly, the adipocytes from some donors were unable to withstand high palmitate concentrations. The most obvious difference between palmitate-tolerant and palmitate-intolerant adipocytes was in their capacity for DNL and SCD-mediated desaturation. The reduction in \textit{DGAT2} mRNA expression and the lower capacity for TG storage seen in the palmitate-intolerant adipocytes may have arisen as a consequence of reduced DNL and SCD activity. Another difference noted between the two groups of adipocytes was their expression of \textit{PCK1} mRNA. A reduction in glycerol 3-phosphate in the palmitate-intolerant adipocytes may have limited the capacity for fatty acid esterification and, hence, TG formation. An inability to store fatty acids as TG in the adipocyte would have knock-on effects. As with lipid storage disorders, such a lipodystrophy, an inability to store circulating fatty acids safely in adipose tissue, could result in ectopic fat deposition in nonadipose tissues (34), a situation shown to have lipotoxic effects on many tissues and linked with metabolic disturbances (35, 36). It appeared that the inability to store fatty acids as TG led to them being redirected toward incorporation into PL fatty acids. There was a higher incorporation of [U-\(^{13}\)C]palmitate in the PL fatty acids of the palmitate-intolerant adipocytes, which may have led to rupturing of the cell membrane.

The SCD loss-of-function studies revealed a down-regulation of the DNL pathway within the adipocyte, confirming their mutual regulation. However, Sampath et al. (37) reported that mice fed a high stearate diet up-regulated \textit{Scd1} prior to other DNL genes such as \textit{Acc}, \textit{Fas}, and \textit{Gpat}. That study measured lipogenic gene expression in liver and was conducted in mice and thus may not mirror the situation in human adipose tissue. In our study, SCD knockdown was also associated with an increase in PL SAFA:MUFA, reduced cell membrane fluidity, and reduced insulin sensitivity of the adipocyte. Improved cell membrane fluidity as a result of increased SCD is thought to be a mechanism employed by cancer cells to avoid apoptosis and increase their survival (38). Furthermore, SAFAs have also been shown to reduce insulin sensitivity (39), stressing the importance of desaturation of SAFAs in cell function. Although there are claims that SCD inhibition may provide a pharmaceutical means to combat obesity (40, 41), this study and others provide evidence that SCD inhibition in the adipocyte may be detrimental to cell function and lead to unwanted side effects (42–44).

The cytotoxic effects of palmitate in other cell types involve induction of inflammatory pathways (45). Activation of AMP-activated protein kinase (AMPK) can inhibit this effect (3, 4, 45), either by increasing fatty acid oxidation or more directly (45). Because AMPK activation would down-regulate DNL, this would seem to be an independent pathway from the one we have described. It could be interesting in future experiments to investigate AMPK activation in adipocytes; if DNL itself were inhibited, but elongation and SCD pathways unaffected, this could be additionally beneficial and might lead to a further rationale for the effectiveness of AMPK-activating drugs such as metformin on insulin sensitivity.

In conclusion, the importance of DNL in the human adipocyte may be as a key regulator of elongation and desaturation, which in turn may assist in the maintenance of cell function. Channeling of DNL-derived palmitate toward elongation and desaturation to produce an endogenous supply of oleate may be necessary for the safe storage of the influx of exogenous palmitate. Consequently, fatty acids may be diverted away from TG storage and instead shunted toward incorporation into plasma membrane phospholipids, which may contribute to a loss of insulin sensitivity. In extreme cases, this may lead to rupturing of the cell membrane. The loss of capacity for TG storage may result in ectopic fat deposition with attendant adverse metabolic consequences.

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REFERENCES

1. Busch, A. K., Gurisik, E., Cordery, D. V., Sudlow, M., Denyer, G. S., Laybutt, D. R., Hughes, W. E., and Biden, T. J. (2005) \textit{Diabetes} \textbf{54}, 2917–2924
2. Moffitt, J. H., Fielding, B. A., Evershed, R., Berstan, R., Currie, J. M., and Clark, A. (2005) \textit{Diabetologia} \textbf{48}, 1819–1829
3. Borradaile, N. M., Han, X., Harp, J. D., Gale, S. E., Ory, D. S., and Schaffer, J. E. (2006) \textit{J. Lipid Res.} \textbf{47}, 2726–2737
4. El-Assaad, W., Buteau, J., Peyot, M. L., Nolan, C., Roduit, R., Hardy, S., Joly, E., Dbaibo, G., Rosenberg, L., and Prentki, M. (2003) \textit{Endocrinology} \textbf{144}, 4154–4163
5. Listenberger, L. L., Han, X., Lewis, S. E., Cases, S., Farese, R. V., Jr., Ory, D. S., and Schaffer, J. E. (2003) Proc. Natl. Acad. Sci. U.S.A. \textbf{100}, 3077–3082
6. Maedler, K., Oberholzer, J., Bucher, P., Spinias, G. A., and Donath, M. Y. (2003) \textit{Diabetes} \textbf{52}, 726–733
7. Summers, L. K., Fielding, B. A., Bradshaw, H. A., Ilic, V., Beysen, C., Clark, M. L., Moore, N. R., and Frayn, K. N. (2002) \textit{Diabetologia} \textbf{45}, 369–377
8. Vessby, B., Unniska, M., Hermansen, K., Riccardi, G., Rivellese, A. A., Tapsell, L. C., Nilsen, C., Berglund, L., Louheranta, A., Rasmussen, B. M., Calvert, G. D., Mattisson, A., Pedersen, E., Gustafsson, I. B., and Storlien, L. H. (2001) \textit{Diabetologia} \textbf{44}, 312–319
9. Chong, M. F., Hodson, L., Bickerton, A. S., Roberts, R., Neville, M., Karpe, F., Frayn, K. N., and Fielding, B. A. (2008) \textit{Am. J. Clin. Nutr.} \textbf{87}, 817–823
10. Saggerson, E. D. (1972) \textit{Biochim. Biophys. Acta} \textbf{315}, 1069–1078
11. Saggerson, E. D., and Tomassi, G. (1971) \textit{Eur. J. Biochem.} \textbf{23}, 109–117
12. Lin, J., Yang, R., Tarr, P. T., Wu, P. H., Handschin, C., Li, S., Yang, W., Pei, L., Uldry, M., Tontonoz, P., Newgard, C. B., and Spiegelman, B. M. (2005) \textit{Cell} \textbf{120}, 261–273
13. Cong, W. N., Tao, R. Y., Tian, J. Y., Liu, G. T., and Ye, F. (2008) \textit{Life Sci.} \textbf{82}, 983–990
