

Individual Stearoyl-CoA Desaturase 1 Expression Modulates Endoplasmic Reticulum Stress and Inflammation in Human Myotubes and Is Associated With Skeletal Muscle Lipid Storage and Insulin Sensitivity In Vivo

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OBJECTIVE—Increased plasma levels of free fatty acids occur in obesity and type 2 diabetes and contribute to the development of insulin resistance. Saturated fatty acids (SFAs) such as palmitate especially have lipotoxic effects leading to endoplasmic reticulum (ER) stress, inflammation, and insulin resistance. Stearoyl-CoA desaturase 1 (SCD1) plays a key role in preventing lipotoxic effects, as it converts SFAs to less harmful monounsaturated fatty acids. Here, we tested the hypothesis that individual differences in the regulation of SCD1 expression by palmitate exist and influence insulin sensitivity and the cellular response to palmitate.

RESEARCH DESIGN AND METHODS—Palmitate-induced gene expression was studied in primary human myotubes of 39 metabolically characterized individuals, as well as in an SCD1-overexpressing cell culture model.

RESULTS—SCD1 mRNA expression and inducibility by palmitate in cultured myotubes showed a broad interindividual variation, presumably due to inheritable characteristics of the donors. Overexpression of SCD1 prevented the inflammatory and ER stress response to palmitate exposure. In primary human myotubes, high SCD1 inducibility was associated with a low inflammatory (interleukin [IL]-6, IL-8, and chemokine [CXC motif] ligand 3 [CXCL3]) and ER stress (CCAAT/enhancer binding protein [C/EBP] homologous protein, activating transcription factor 3 [ATF3], and X-box binding protein 1 [XBP1]) response to palmitate exposure. Finally, palmitate-stimulated SCD1 mRNA expression, positively correlated with intramyocellular lipid (IMCL) content of the donors, was measured by ¹H-magnetic resonance spectroscopy. After adjustment for IMCL, SCD1 expression and inducibility were positively correlated with insulin sensitivity.

CONCLUSIONS—We hypothesize that myocellular SCD1 inducibility by palmitate is an individual characteristic that modulates lipid storage, palmitate-induced inflammation, ER stress, and insulin resistance. This may describe individuals with increased capability of innocuous free fatty acid handling and benign triglyceride storage. *Diabetes* 58:1757–1765, 2009

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Published ahead of print at <http://diabetes.diabetesjournals.org> on 28 May 2009. DOI: 10.2337/db09-0188.

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Diabetes and the metabolic syndrome represent an increasing problem worldwide. In obesity and type 2 diabetes, elevated plasma concentrations of nonesterified free fatty acids (FFAs) are observed (1,2). The increased release of FFAs from the adipose tissue is an important factor modulating insulin sensitivity. Insulin resistance can develop within hours of an acute elevation of plasma FFA levels in humans (2,3). Proposed mechanisms leading to FFA-induced insulin resistance are summarized under the concept of lipotoxicity. Lipotoxicity involves an increase of intracellular fatty acid metabolites such as diacylglycerol and ceramides, leading to endoplasmic reticulum (ER) stress and serine/threonine phosphorylation of insulin receptor substrates and the activation of nuclear factor (NF)- κ B signaling pathways (1). A consecutive acute inflammatory response with secretion of cytokines and diminished events downstream of insulin receptor signaling cascade leads to a low-grade inflammatory state associated with insulin resistance.

The saturated fatty acids (SFAs) stearate and palmitate especially have a strong lipotoxic potential to induce inflammation, ER stress, and insulin resistance (4–8). The microsomal enzyme stearoyl-CoA desaturase 1 (SCD1) plays a key role in modulating these effects of SFAs (8–11). SCD1 desaturates stearate and palmitate to generate the less toxic monounsaturated fatty acids (MUFAs) oleate and palmitoleate. SCD1 activity is subject to a complex and tight regulation by hormonal and nutrient stimuli on the transcriptional level (10,12–14). While polyunsaturated fatty acids and fasting strongly suppress SCD1 expression, insulin, glucose, and SFAs potentially stimulate SCD1 expression on a transcriptional level. Overexpression of SCD1 promotes triglyceride storage and reduces palmitate-induced apoptosis and ceramide and diacylglycerol synthesis, as well as insulin resistance (9–11). In vivo, increased skeletal muscle SCD1 expression is observed in conditions with elevated plasma FFA levels like obesity or after physical exercise (9,15–17). As SCD1 activity prevents lipotoxicity in cell culture models (9–11), upregulated SCD1 expression in response to the SFA palmitate could be an endogenous protective mechanism to ameliorate its deleterious effects. In the present study, we tested the hypothesis that interindividual differences in upregulation of SCD1 expression in response to palmitate expo-

sure might exist that would modulate effects of FFAs in humans. Therefore, we studied individual palmitate-induced SCD1 gene expression in primary human myotubes of 39 metabolically characterized individuals and studied the relation with intramyocellular lipid (IMCL) content and insulin sensitivity. Furthermore, we evaluated the effect of individual SCD1 expression on markers of inflammation and ER stress.

RESEARCH DESIGN AND METHODS

A total of 39 individuals were studied. These subjects were healthy nondiabetic participants of the Tübingen Family Study for Type 2 Diabetes (TÜF study) and gave informed written consent to the study. Individuals were recruited from the southern part of Germany and were not related to each other. The participants did not take any medication known to affect glucose tolerance or insulin sensitivity. The ethical committee of the Tübingen University Medical Department had approved the protocol.

Determination of blood parameters. Plasma glucose was determined using a bedside glucose analyzer (glucose oxidase method; Yellow Springs Instruments, Yellow Springs, OH). Serum FFA concentrations were measured with an enzymatic method (Wako Chemicals, Neuss, Germany). Plasma triglycerides, total cholesterol, and HDL and LDL cholesterol were measured using the ADVIA 1650 clinical chemical analyzer, and insulin was analyzed using the ADVIA Centaur immunoassay system according to the instructions of the manufacturers. Both analyzers were from Siemens Medical Solutions (Fernwald, Germany).

Insulin sensitivity. After a 10-h overnight fast, all subjects underwent a 75-g oral glucose tolerance test, as previously described (18,19).

Determination of IMCL by magnetic resonance spectroscopy. Neutral lipids within the muscle cell (IMCL) and those interlaced between the muscle fibers can be differentiated due to their geometrical arrangement using proton magnetic resonance spectroscopy. Localized image-guided proton spectra of the tibialis anterior muscle were acquired on a 1.5-Tesla whole-body imager (Magnetom Vision; Siemens, Erlangen, Germany). For volume selection, a single-voxel STEAM technique was applied. Measurement parameters were echo time, 10 msec; repetition time, 2 s; volume of interest, $11 \times 11 \times 20 \text{ mm}^3$; and 40 acquisitions. IMCL in the tibialis muscle were quantified as previously described (20).

Body composition. Total body fat and lean body mass were measured by bioelectrical impedance (RJL, Detroit, MI).

Measurement of $\dot{V}O_{2\text{max}}$. The individuals underwent a continuous, incremental exercise test to volitional exhaustion using a cycle ergometer. The cycle ergometer test was performed on an electromagnetically braked cycle ergometer (Ergometrics 800 S; Ergoline, Bitz, Germany). Oxygen uptake was measured using a spiroergometer (MedGraphics System Breese Ex 3.02 A; MedGraphics, St. Paul, MN). Maximal aerobic capacity was expressed as $\dot{V}O_{2\text{max}}$ (milliliters per minute) per kilogram lean body mass.

Preparation of BSA bound FFAs. In most experiments, cells were incubated with the FFAs palmitate or oleate. FFAs were bound to fatty acid-free albumin solution from bovine serum fraction V 10% in sterile filtered Dulbecco's PBS (Sigma-Aldrich, Taufkirchen, Germany). In brief, FFAs (200 mmol/l in ethanol) were diluted in the BSA solution to a concentration of 6 mmol/l (the FFA-to-BSA ratio was 4 to 1 mol to mol). This mixture was gently agitated at 37°C under nitrogen overnight. These stock solutions were stored in aliquots under nitrogen at -20°C .

Cell culture. Primary human skeletal muscle cells were obtained from needle biopsies of the vastus lateralis muscle. The primary skeletal muscle cells were grown and differentiated as previously described (21). When myoblasts reached 80–90% confluence, the cells were fused for 4 days in α -minimum Eagle's medium containing 5.5 mmol/l glucose with 0.2% antibiotic antimycotic solution (Invitrogen) and 2% fetal bovine serum (fusion medium). On day 5, myotubes were stimulated with FFAs (0.5 mmol/l) or BSA for 20 h in fusion medium. Human embryonic kidney (HEK) 293 cells were stably transfected with a hemagglutinin (HA)-tagged human SCD1 expression vector (10) or the empty control vector pCDNA3 and cultured in DMEM (BioWhittaker, Verviers, Belgium) containing nonessential amino acids supplemented with 2 mmol/l glutamine, 1 mmol/l pyruvate, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 0.5 mg/ml geneticin, and 10% FCS.

Cell viability assay. The water soluble tetrazolium (WST)-1 viability assay (Roche Molecular Biochemicals, Mannheim, Germany) was used to determine HEK293 cell survival after palmitate exposure according to the manufacturer's instructions. The amount of formazan dye formed directly correlates to the number of metabolically active and viable cells in the culture. A total of 3,000 cells were seeded per well in 96-well plates the day before treatment. Cell viability was analyzed 48 h after addition of BSA or palmitate (0.6 mmol/l).

BSA-treated cells were set as 100% viability, and cell survival (%) was determined by averaging four repeated experiments performed in quadruplicate.

Real-time RT-PCR. Cells were treated for 20 h, subsequently washed, and harvested. RNA was isolated with RNeasy columns (Qiagen, Hilden, Germany). Quality and quantity of total RNA was assessed spectrophotometrically. Total RNA treated with RNase-free DNase I was transcribed into cDNA using AMV reverse transcriptase and the first-strand cDNA kit from Roche Diagnostics (Mannheim, Germany). Quantitative PCR was performed at least in duplicate with SYBR Green on a LightCycler (Roche Diagnostics).

Conditions for RT-PCR quantification of mRNAs. The conditions for RT-PCR quantification of mRNAs were as follows: hSCD1 5'-TGCAGGAC-GATATCTCTAGC-3', 5'-ACGATGAGCTCCTGTGTGTA-3', T 58°C, 45 cycles, 3 mmol/l MgCl₂; interleukin (IL)-8 5'-AAGAAACCACCGAAGGAAC-3', 5'-ACT-TCTCCACAACCCTCTGC-3', T 68°C, 45 cycles, 3 mmol/l MgCl₂; IL-6 5'-CCAGCTATGAACTCCTCTC-3', 5'-GCTTGTTCCTCACATCTCTC-3', T 63°C, 45 cycles, 3 mmol/l MgCl₂; chemokine (CXC motif) ligand 3 (CXCL3) 5'-CTGCCCTTACCAGAGCTGAA-3', 5'-TCCCCACCCTGTTCATTTA TC-3', Tan 61°C, 45 cycles, 4 mmol/l MgCl₂; and XBP1s 5'-CCGCAGCAGGTGCAGG-3', 5'-GGGGCTTGGTATATATGTGG-3', T 66°C, 3 mmol/l MgCl₂ (22).

QuantiTect primer assays. Activating transcription factor 3 (ATF3), CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), and X-box binding protein 1 (XBP1) RT-PCRs were performed using a QuantiTect SYBR Green PCR Kit and QuantiTect Primer Assays Hs_ATF3_1_SG QT00000273, Hs_DDIT3_1_SG (QT00082278), and Hs_XBP1_1_SG (QT00068383) according to the manufacturer's instructions (Qiagen, Hilden, Germany) on a Roche LightCycler 480 (Mannheim, Germany).

Cellular fatty acid composition. The total cellular fatty acids were subjected to direct transesterification. The quantification of the fatty acid methyl ester composition was analyzed using gas chromatography with a flame ionization detector (7890A GC system; Agilent Technologies, Waldbronn, Germany) on a 60 m \times 0.25 mm \times 0.2 μm fused silica column (Rtx 2330; Rystek) as previously described (10).

Western blotting. Cytosolic cell extracts were separated by SDS-PAGE, and proteins were transferred to a nitrocellulose membrane by semidry electroblotting. Immunodetection was performed using a mouse monoclonal antibody against the full-length recombinant human hSCD1 (ab19862) (Abcam, Cambridge, U.K.) and the cytosolic protein glutamine:fructose-6-phosphate aminotransferase (23).

Calculations and statistics. Insulin sensitivity was estimated from the oral glucose tolerance test (in arbitrary units) as proposed by Matsuda and DeFronzo (24) $[10,000/(\text{Glc}_0 \times \text{Ins}_0 \times \text{Glc}_{\text{mean}} \times \text{Ins}_{\text{mean}})^{1/2}]$. *P* values were determined by Student's *t* test on log-transformed data of non-normally distributed parameters. To adjust insulin sensitivity for the independent effects of IMCL or BMI, we performed multivariate regression analysis. The statistical software package JMP 4.0 (SAS Institute, Cary, NC) was used.

RESULTS

First, we examined the effect of palmitate exposure on SCD1 mRNA expression in primary myotubes of 39 donors. The anthropometric and metabolic characteristics of the myotube donors are displayed in Table 1. On average, we observed a doubling of SCD1 mRNA expression after palmitate exposure (0.5 mmol/l, 20 h). However, basal SCD1 mRNA expression levels, as well as the upregulation of SCD1 mRNA expression in response to palmitate, showed a remarkable interindividual variation. In myotubes of three donors, the SCD1 expression was reduced, while most others showed a strong but variable increase (0.46- to 4.38-fold) (Fig. 1A and B).

Next, we established a cell culture model to study the role of SCD1 in fatty acid-induced inflammation and ER stress. We generated a cell line with stable overexpression of HA-tagged human SCD1 in HEK293 cells. In the Western blot, overexpressed SCD1-HA can be detected above the endogenous SCD1 protein due to the added HA tag (Fig. 2A). Fatty acid analysis revealed a significantly higher proportion of the MUFA (C16:1) and the SCD1 product-to-substrate ratio (C16:1/C16:0) in SCD1-overexpressing cells compared with controls (Fig. 2B). As overexpression of SCD1 has been shown to prevent lipotoxicity (10,11), the

TABLE 1
Anthropometric and metabolic data from myotube donors and the subgroup with available data on IMCLS

Demographics and body composition	All participants	Participants with IMCL data
Sex (female/male)	18/21	10/18
Age (years)	26 ± 5 (19–36)	27 ± 4 (22–35)
BMI (kg/m ²)	23.3 ± 3.5 (18.3–35.7)	22.8 ± 2.8 (18.3–29.1)
Body fat _{bioimpedance} (%)	21.9 ± 8.1 (7–39)	19.7 ± 7.6 (7–37)
Waist-to-hip ratio	0.81 ± 0.08 (0.7–1)	0.81 ± 0.08 (0.7–1)
SCD1 induction by palmitate (fold)	1.95 ± 0.91 (0.46–4.38)	1.96 ± 0.86 (0.53–4.38)
IMCL _{MRS} (AU)		3.4 ± 1.7 (0.5–7.8)
Metabolic characteristics		
Fasting glucose (mmol/l)	4.8 ± 0.43 (3.8–5.4)	4.7 ± 0.41 (3.9–5.4)
2-h glucose (mmol/l)	5.27 ± 1.1 (3.5–7.4)	5.17 ± 1.1 (3.5–7.1)
Fasting insulin (pmol/l)	38.3 ± 20 (13–103)	38.2 ± 19 (13–79)
2-h insulin (pmol/l)	189.9 ± 114 (60–524)	189.9 ± 111 (60–491)
Fasting serum FFAs (μmol/l)	408 ± 167 (140–808)	414 ± 179 (163–808)
Fasting triglycerides (mg/dl)	88.9 ± 37 (34–186)	83.8 ± 32 (43–186)
Fasting total cholesterol (mg/dl)	183 ± 38 (119–299)	182 ± 40 (123–299)
Fasting HDL cholesterol (mg/dl)	56.1 ± 15 (37–96)	57.1 ± 14 (38–96)
Fasting LDL cholesterol (mg/dl)	114.3 ± 33 (63–207)	113.6 ± 34 (63–207)
V _{O₂Max} (ml · min ⁻¹ · kg lbm ⁻¹)		41.8 ± 15 (22.3–95.6)
ISI _{OGTT} (AU)	26.2 ± 10.9 (6.5–47)	25.9 ± 10.9 (6.5–47)

Data are means ± SE (range). ISI_{OGTT}, insulin sensitivity estimated from the OGTT; lbm, lean body mass; MRS, magnetic resonance imaging; OGTT, oral glucose tolerance test.

SCD1-overexpressing cells showed increased viability in a WST-1 assay (+42%, $P = 0.007$) after extended exposure to palmitate (0.6 mmol/l, 48 h). These results indicate a detoxifying effect of overexpressed SCD1 in our cell model.

After having characterized the SCD1 cell line, we determined the induction of inflammatory cytokines and key ER stress markers upon palmitate and oleate exposure (0.5 mmol/l, 20 h). Palmitate strongly induced the mRNA expression of the inflammatory cytokines IL-8 (17-fold) and CXCL3 (3.6-fold) in control cells but not in SCD1-overexpressing cells (Fig. 3A and B). Oleate, a MUFA and

a product of SCD1 action, did not induce these inflammatory cytokines. IL-6 mRNA was below detection limits in these cells by RT-PCR. Next, we investigated palmitate-induced ER stress, which is a potential molecular link between obesity, elevated FFAs, insulin resistance, and the progression to diabetes (1). The ER stress markers CHOP, ATF3, XBP1, and the spliced form XBP1s were also significantly induced after palmitate exposure (Fig. 3C–F). In contrast, oleate did not induce expression of the ER stress markers. The induction of inflammatory and ER stress markers IL-8, CXCL3, ATF3, XBP1, and XBP1s was almost completely prevented in SCD1-overexpressing cells (Fig. 3A–F). These experiments demonstrate that SCD1 overexpression can prevent palmitate-induced inflammatory response, ER stress, and lipotoxicity.

Following this, we aimed to validate these findings in our set of primary human myotubes. We tested the hypo-

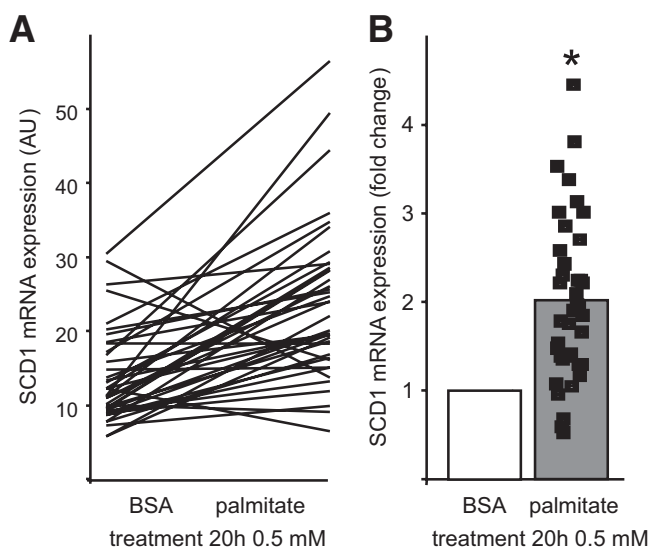


FIG. 1. SCD1 mRNA expression is stimulated by the saturated fatty acid palmitate. **A:** Change in SCD1 mRNA expression in primary human myotubes of 39 donors is displayed after 20 h exposure to 0.5 mmol/l palmitate or the BSA control. **B:** Fold changes of SCD1 expression after palmitate exposure are displayed. The induction of SCD1 expression ranges from 0.46- to 4.38-fold. SCD1 mRNA expression is stimulated 1.95-fold by palmitate exposure. * $P < 0.0001$ (significantly different).

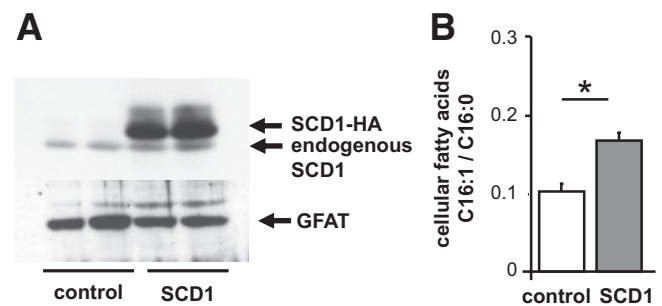


FIG. 2. Characterization of an SCD1 overexpression model in HEK293 cells. HEK293 cells were stably transfected with HA-tagged human SCD1. **A:** Endogenous SCD1 and overexpressed SCD1-HA protein expression is visualized by Western blotting using an anti-human-SCD1 antibody. The strongly overexpressed SCD1-HA is visible above the endogenous SCD1. The cytosolic protein glutamine:fructose-6-phosphate aminotransferase was used as a loading control. **B:** Analysis of the cellular fatty acid composition revealed a significantly higher fatty acid ratio of SCD1 product to substrate (C16:1/C16:0) in SCD1 than in control cells. Means ± SE of $n = 4$ are displayed. *Significantly different between the groups, $P < 0.01$.

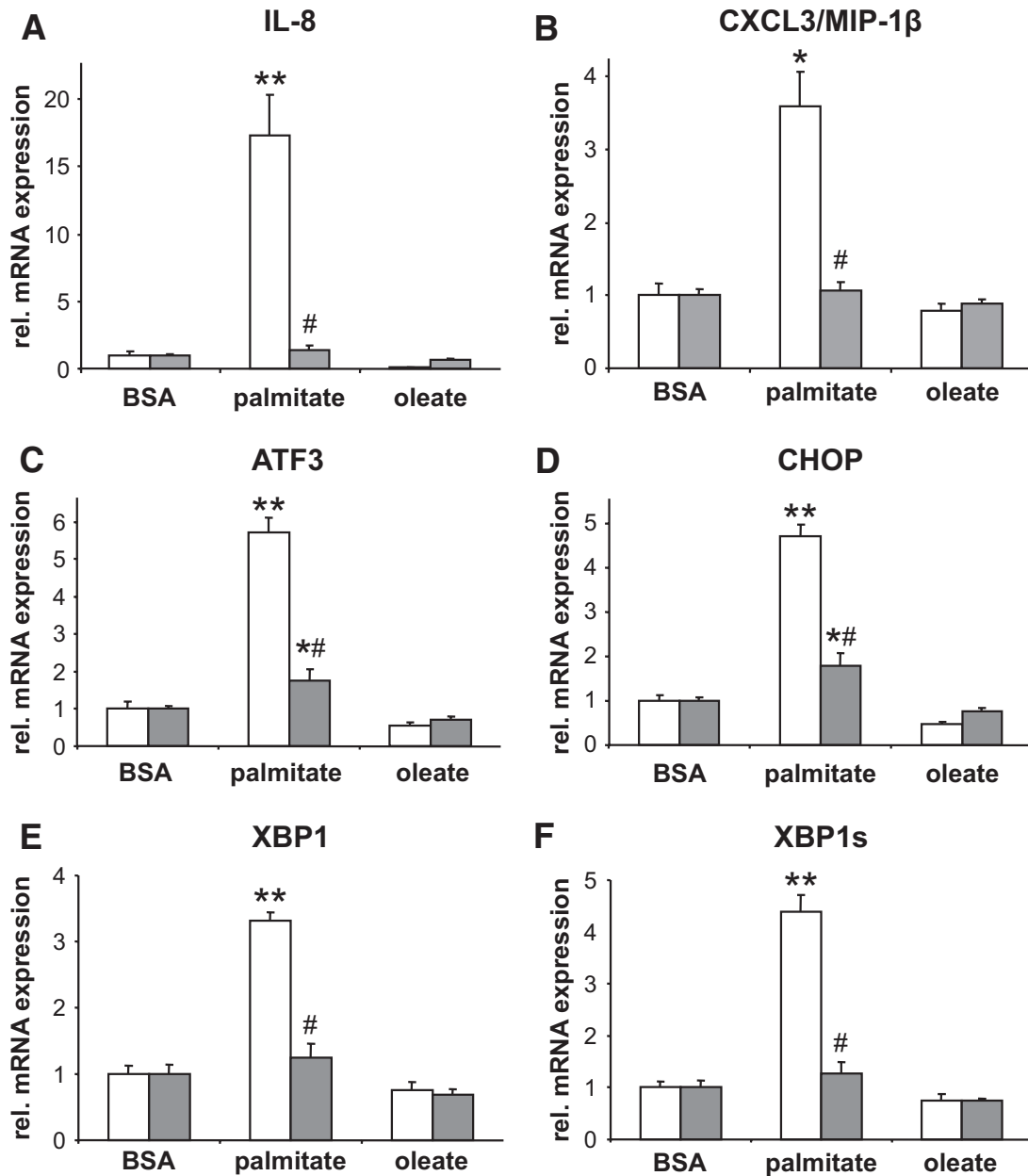


FIG. 3. Overexpression of SCD1 prevents palmitate-induced inflammatory cytokine induction and ER stress. The expression of inflammatory cytokines (A and B) and ER stress response markers (C–F) in response to palmitate or oleate (0.5 mmol/l; 20 h). Exposure in SCD1 cells and control cells is displayed. Palmitate, but not oleate, induces the expression of inflammatory cytokines. Overexpression of SCD1 almost completely prevents the induction of IL-8 and CXCL3 by palmitate. The ER stress response markers ATF3, CHOP, XBP1, and the spliced form XBP1s are increased by palmitate, but not oleate, exposure. Overexpression of SCD1 almost completely prevents the induction of ATF3, CHOP, XBP1, and the spliced form XBP1s by palmitate. Means \pm SE of $n = 6$ are displayed. *Significantly different palmitate vs. BSA exposure, $P < 0.05$; ** $P < 0.001$; #Significantly different SCD1 vs. control cells, $P < 0.001$. \square , control; \blacksquare , SCD1.

esis that interindividual differences in SCD1 gene expression modulate cellular effects of palmitate exposure, such as induction of inflammation and ER stress. Therefore, myotube donors were divided in a low and a high SCD1 group according to the mean SCD1 inducibility by palmitate in the differentiated myotube cell cultures (Fig. 1). The two donor groups did not differ in age, BMI, waist-to-hip ratio, body fat %, Vo_{2max} , fasting triglycerides and FFA levels, or insulin sensitivity (data not shown).

Exposure to palmitate caused a strong induction of inflammatory cytokines IL-6 (13-fold), IL-8 (12-fold), and CXCL3/macrophage inflammatory protein (MIP)-1 β (17-fold) mRNA expression in human myotubes of all donors. The induction of all three inflammatory cytokines upon

palmitate exposure was significantly lower in the high SCD1 group compared with the low SCD1 group (Fig. 4A–C). This further indicates that high individual inducibility of SCD1 reduces the cellular inflammatory response to palmitate.

Next, we investigated the expression of palmitate-induced ER stress markers. Palmitate exposure induced the expression of key ER stress markers CHOP (4.7-fold) and ATF3 (5.3-fold) in myotubes of all donors. XBP1 and the activated spliced form XBP1s (not shown) showed a less pronounced induction (1.7- and 1.6-fold, respectively). We could demonstrate that the induction of CHOP and ATF3 was significantly reduced in the high-SCD1 group (Fig. 4D and E). XBP1 also tended to have a less pro-

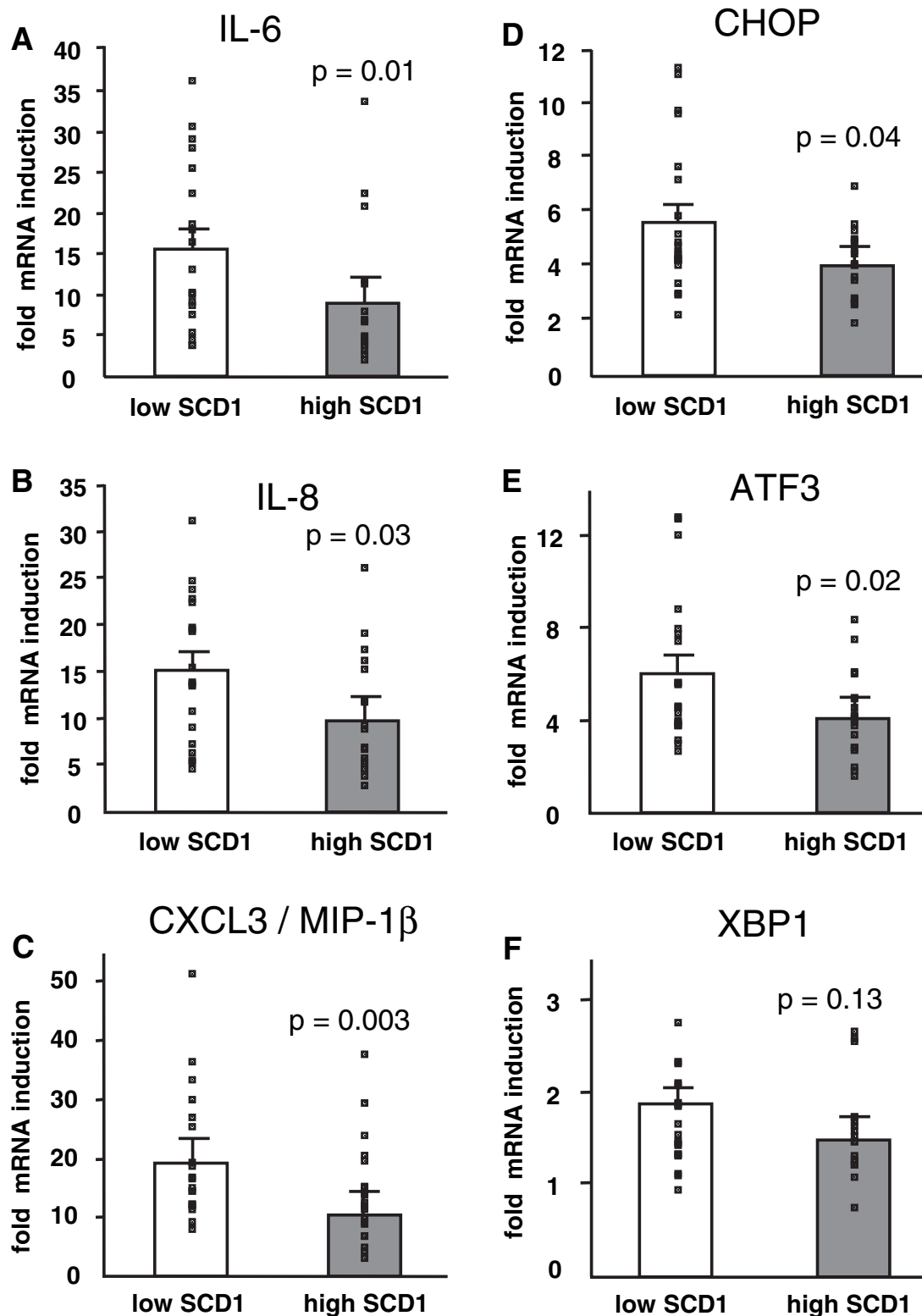


FIG. 4. High inducibility of SCD1 is associated with a low inflammatory and ER stress response to palmitate in human myotubes. Myotube donors were divided by the mean of SCD1 induction by palmitate into a group with low (\square) and high (\blacksquare) inducibility of SCD1. Induction of inflammatory cytokines IL-6, IL-8, and CXCL3/MIP-1 β by palmitate was compared between the low and high SCD1 group. The high SCD1 group had significantly lower induction of inflammatory cytokines IL-6 (15.7- vs. 8.9-fold, $P = 0.01$) (A), IL-8 (15.0- vs. 9.9-fold, $P = 0.03$) (B), and CXCL3 (20.0- vs. 11.4-fold, $P = 0.003$) (C). The induction of key ER stress markers by palmitate was also compared between the low and high SCD1 group. The high SCD1 group had significantly lower induction of CHOP (5.53- vs. 3.99-fold, $P = 0.04$) (D) and ATF3 (6.02- vs. 4.05-fold, $P = 0.02$) (E) and tended to lower induction of XBP1 (1.86- vs. 1.52-fold, $P = 0.13$) (F).

nounced increase in the high-SCD1 group, although it did not reach significance (Fig. 4F). These results demonstrate that high individual inducibility of SCD1 mRNA reduces

palmitate-induced ER stress and further support that the individual inducibility of SCD1 is an important factor to prevent lipotoxic effects of palmitate. Following this, we

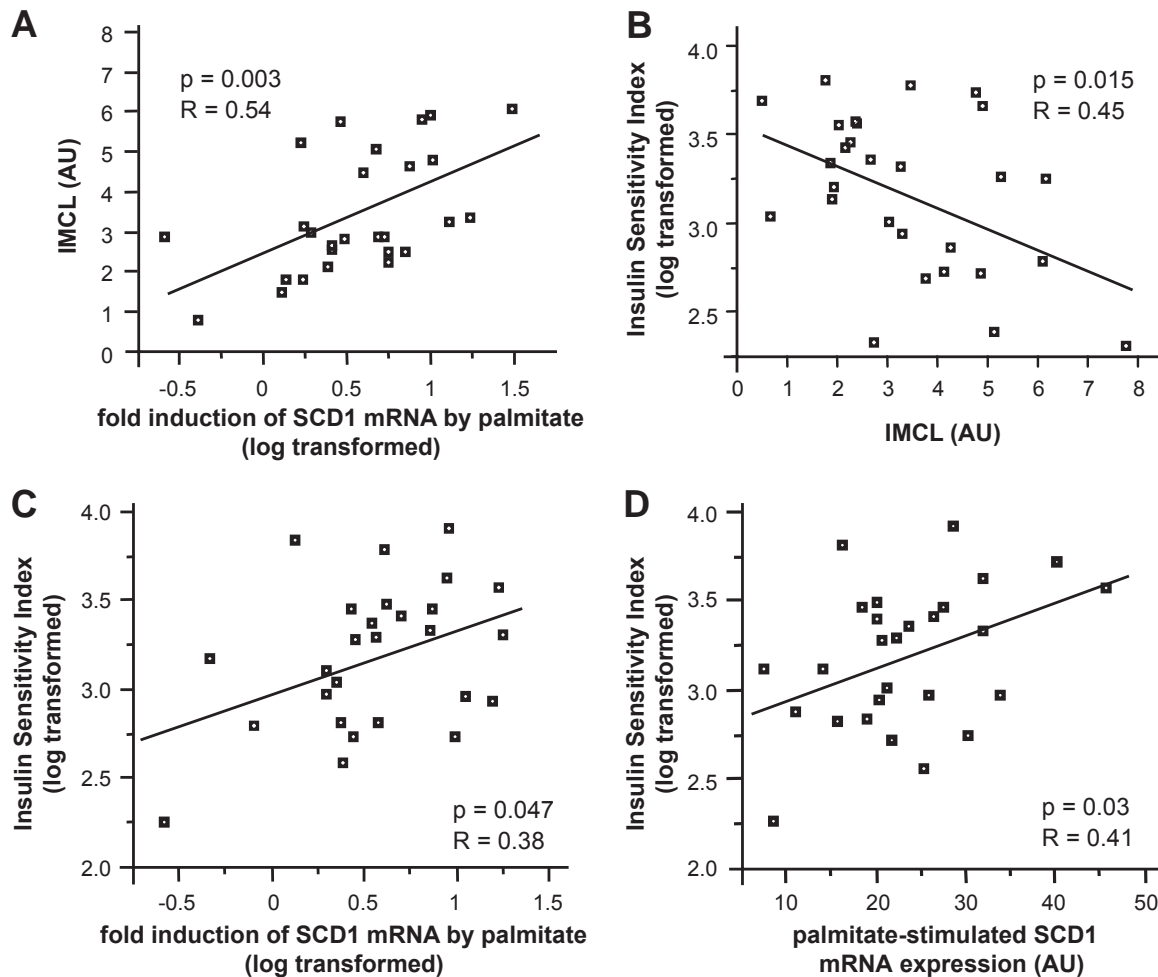


FIG. 5. Relationship between SCD1 expression in vitro, the donors' IMCL levels, and insulin sensitivity. **A:** The relative increase of SCD1 mRNA expression in response to palmitate exposure is positively correlated with the donors' IMCL ($n = 28$, adjusted for BMI). **B:** Relationship between IMCL and insulin sensitivity, estimated from the OGTT (24). In the studied population, insulin sensitivity was negatively correlated with IMCL adjusted for BMI. **C and D:** Relationship between insulin sensitivity and SCD1 expression cultured myotubes. The relative induction of SCD1 mRNA expression by palmitate (**C**) as well as absolute SCD1 mRNA expression levels after palmitate exposure (**D**) was positively correlated with the donors' insulin sensitivity after adjustment for IMCL.

turned to the myotube donors and investigated if SCD1 expression in vitro describes an individual characteristic that also influences lipid metabolism in vivo.

As the mechanism of how SCD1 prevents lipotoxicity involves triglyceride storage (11), we investigated the relation between SCD1 mRNA induction in myotubes with IMCL levels and insulin sensitivity of the donors in vivo. IMCL levels were assessed by noninvasive ^1H -magnetic resonance spectroscopy in a subgroup of 28 subjects. SCD1 inducibility ($R = 0.54$, $P = 0.003$, adjusted for BMI) (Fig. 5A) as well as palmitate-stimulated SCD1 mRNA expression levels ($R = 0.42$, $P = 0.03$, not shown) determined in in vitro-differentiated myotubes was positively correlated with IMCL levels in the tibialis muscle of the myotube donors. This demonstrates an in vivo relevance of individual SCD1 gene expression for skeletal muscle lipid storage. No correlation between the donors' BMI, waist-to-hip ratio, or plasma lipid levels and SCD1 expression in vitro was observed. As reported previously in multiple studies (25,26), higher IMCL levels are correlated with insulin resistance in this group ($R = 0.45$, $P = 0.015$) (Fig. 5B). However, insulin sensitivity was not correlated with SCD1 expression or inducibility ($R = 0.08$, $P = 0.64$; $R = 0.10$, $P = 0.71$, respectively, not shown). To investi-

gate the influence of SCD1 expression on insulin sensitivity independent of IMCLs we also performed this analysis after adjustment for IMCLs. Insulin sensitivity was positively correlated with SCD1 inducibility ($R = 0.38$, $P = 0.047$) (Fig. 5C) as well as SCD1 expression levels after palmitate stimulation ($R = 0.41$, $P = 0.03$) (Fig. 5D). Thus, SCD1 inducibility might have beneficial effects for the maintenance of insulin sensitivity.

DISCUSSION

SCD1 is a key regulator in lipid metabolism and modulates cellular effects of SFAs. In the present study, we could show that interindividual differences of SCD1 inducibility by palmitate exist in human myotubes. After the extended culture protocol of myoblast isolation and myotube differentiation, environmental and humoral factors from the donors are a priori excluded. Therefore, differences in SCD1 gene expression or regulation are most likely due to the donors' genetic or epigenetic characteristics (15,21), which can influence both gene expression in cell culture and skeletal muscle lipid metabolism in vivo. The molecular mechanism of how SFAs induce SCD1 expression is not fully understood but may involve activation of NF- κ B

through the toll-like receptor 2 (TLR2) pathway and binding to NF- κ B-responsive elements in the SCD1 promoter as well as fatty acid upregulation of peroxisome proliferator-activated receptor- γ coactivator 1 β and subsequent coactivation of sterol regulatory element-binding protein-1c (14,27–29). In the present study, we show that SCD1 inducibility displays an immense interindividual variation and is a determinant of the individuals' lipid metabolism. We hypothesized that upregulation of SCD1 expression in response to palmitate exposure represents a protective mechanism against lipotoxicity, and individual differences could influence the susceptibility for deleterious effects of SFAs.

Palmitate exposure induced an inflammatory response in primary human skeletal muscle cells as well as in HEK cells. In our experiments, overexpression of SCD1 in HEK cells completely prevented the inflammatory response to palmitate exposure. Consistently, we could show that high SCD1 inducibility is associated with low inflammatory response to palmitate exposure and is associated with reduced expression of the inflammatory cytokines IL-6, IL-8, and CXCL3 in human myotubes. Murine data support the protective role of SCD1 in lipid-induced inflammation (14,30). SCD1 knockout mice on a MUFA-depleted diet display an inflammatory and ER stress gene expression profile (30). Increased levels of ceramides, which are involved in lipotoxicity and insulin resistance, are observed in hepatic SCD1 knockout mice on a MUFA-free diet (31). Additionally, SCD1 has been linked to inflammation, as downregulation of hepatic SCD1 exacerbates inflammation and acute colitis (32) and absence of SCD1 promotes inflammation and atherosclerosis in a mouse model of familial hypercholesterolemia (33).

The second deleterious effect of palmitate on human skeletal muscle cells is the induction of ER stress, which is discussed as a molecular link between obesity, elevated FFAs, and insulin resistance (1,3,22). ER stress describes the accumulation of misfolded proteins that aggregate in the ER. ER stress activates the unfolded protein response pathways in order to restore ER homeostasis, reduce ER stress, and circumvent cell death. These responses include the splicing of XBP1 mRNA as well as increased XBP1 mRNA transcription and the activation of the PKR-like ER kinase (PERK)/ATF4 pathway that finally leads to the transcriptional activation of the C/EBP homologous protein CHOP and ATF3. Therefore, increasing mRNA levels of XBP1s, XBP1, CHOP, and ATF3 represent markers of ER stress in mammalian cells (1,22).

In human myotubes, palmitate induced increased mRNA expression levels of ATF3, CHOP, XBP1, and activated, spliced XBP1s. We could demonstrate that the induction of ER stress markers was completely prevented by SCD1 overexpression in HEK cells, and high SCD1 inducibility in myotubes was associated with reduced ER stress response. Loss of SCD1 has been shown to increase ER stress (8,30). In several animal models, induction of ER stress is associated with insulin resistance, and chaperones that reduce ER stress have been shown to restore insulin signaling (1,34–37).

As inflammation and ER stress can both be a cause of insulin resistance (1,3) and SCD1 overexpression in skeletal muscle cells prevents palmitate-induced insulin resistance (9), it is suggestive to also expect a protective effect of SCD1 inducibility against insulin resistance *in vivo*. However, at first, we could not detect an association of SCD1 induction with insulin resistance. SCD1 inducibility

by palmitate in myotubes was positively correlated with the donors' IMCL content *in vivo*. This association is well in line with mouse and molecular *in vitro* data showing that SCD1 is essential for triglyceride synthesis and storage (29,38,39). In fact, triglyceride synthesis has been implicated as the crucial mechanism to prevent lipotoxicity (11).

However, multiple studies (25,26) have identified high IMCL as an indicator of insulin resistance in humans. This correlation only exists in lean, sedentary young adults (25,26), like the group described here, but is lost if a general population is studied. In fact, in physically trained subjects, high IMCL is even associated with improved insulin sensitivity (20,40). It is therefore an unsolved question as to whether a causal direct relation between IMCL and insulin resistance exists at all or if both are just parallel consequences of altered skeletal muscle lipid metabolism (41). Based on our data, we speculate that triglyceride storage, as well as inflammation, ER stress, and insulin resistance, may be the common consequence of an increased fatty acid flux into the skeletal muscle cell. Furthermore, high SCD1 activity may funnel fatty acids away from deleterious events toward efficient and innocuous triglyceride storage and prevent insulin resistance. After adjustment for IMCLs, when comparing individuals with equal IMCL levels, SCD1 induction and palmitate-induced SCD1 expression levels were positively related with insulin sensitivity. These findings imply that high inducibility of SCD1 by palmitate facilitates benign triglyceride storage in human skeletal muscle, which does not lead to inflammation, ER stress, and insulin resistance. High SCD1 activity may therefore be a factor that dissociates benign from malign IMCL storage, a concept that has recently been suggested for human obesity (19).

Conflicting data on the role of SCD1 in mediating insulin sensitivity exist from cell culture models and SCD1-deficient mice. SCD1-deficient mice are protected from diet-induced insulin resistance and obesity and have increased lipid oxidation and thermogenesis (14). However, it remains controversial which organ's SCD1 activity (liver, skeletal muscle, or skin) has the major effect for this phenotype of the whole-body SCD1-deficient mice, since liver-specific knockout mice are not protected from high-fat diet-induced obesity and insulin resistance (31,42). It has been suggested that elevated activity of SCD1 partitions fatty acids away from oxidation toward storage of triglycerides (15), a hypothesis that is largely supported by the data presented in this study. Increasing β -oxidation via activation of AMP-activated protein kinase (AMPK) has been implicated as an alternative way of triglyceride storage of innocuous fatty acid handling and preventing lipotoxicity (8,43,44). This pathway, activated by exercise or antidiabetic drugs like metformin, has been shown to reduce palmitate as well as cytokine-induced transactivation of NF- κ B and vascular inflammation in endothelial cells (43). Activation of AMPK was therefore proposed as a target to reduce lipotoxicity and insulin resistance (44,45). As AMPK was found to be activated in multiple tissues of SCD1-deficient mice (46,47), this may be a compensatory mechanism to prevent lipotoxicity and insulin resistance in the absence of SCD1.

In conclusion, we could show that upregulation of SCD1 in response to palmitate has an interindividual variability that is retained in cultured human myotubes and is associated with IMCL content and insulin sensitivity *in vivo*. High SCD1 inducibility represents a protective mechanism

against lipotoxicity and reduces the susceptibility of myotubes toward ER stress and inflammation upon palmitate exposure. This may describe individuals with increased capability of innocuous FFA handling and triglyceride storage.

ACKNOWLEDGMENTS

The studies were supported by grants from the Deutsche Forschungsgemeinschaft (KFO 114, GRK 1302/1, Heisenberg Grant to N.S. [STE 1096/1-1]) and the European Community's FP6 EUGENE2 (LSHM-CT-2004-512013). No other potential conflicts of interest relevant to this article were reported.

We gratefully acknowledge the excellent technical assistance of Iris Mertens and Carina Haas. We thank all study participants for their cooperation.

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