Induction of stearoyl-CoA desaturase protects human arterial endothelial cells against lipotoxicity

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Peter A, Weigert C, Staiger H, Rittig K, Cegan A, Lutz P, Machicao F, Häring H-U, Schleicher E. Induction of stearoyl-CoA desaturase protects human arterial endothelial cells against lipotoxicity. Am J Physiol Endocrinol Metab 295: E339–E349, 2008. First published June 3, 2008; doi:10.1152/ajpendo.00022.2008.—Endothelial lipotoxicity has been implicated in the pathogenesis of multiple stages of cardiovascular disease from early endothelial dysfunction to manifest atherosclerosis and its complications. Saturated free fatty acids are the major inducers of endothelial cell apoptosis and inflammatory cytokines. In humans, the enzyme human stearoyl-CoA desaturase-1 (hSCD-1) is the limiting step of the desaturation of saturated to monounsaturated fatty acids. Since we could demonstrate the expression of SCD-1 in primary human arterial endothelial cells (HAECs), we aimed to prove a beneficial role of upregulated hSCD-1 expression. In contrast to other cells that are less susceptible to lipotoxicity, hSCD-1 was not upregulated in HAECs upon palmitate treatment. Following that, we could show that upregulation of hSCD-1 using the LXR activator TO-901317 in HAECs protects the cells against palmitate-induced lipotoxicity, cell apoptosis, and expression of inflammatory cytokines IL-6 and IL-8. Increased hSCD-1 activity was determined as increased C16:1/16:0 ratio and enhanced triglyceride storage in palmitate treated cells. The beneficial effect was clearly attributed to enhanced hSCD-1 activity. Overexpression of hSCD-1 blocked palmitate-induced cytotoxicity, and knockdown of hSCD-1 using siRNA abolished the protective effect of TO-901317 in HEK-293 cells. Additionally, inhibition of hSCD-1 with 10/12 CLA blocked the effect of TO-901317 on palmitate-induced lipotoxicity, cell apoptosis, and inflammatory cytokine induction in HAECs. We conclude that upregulation of hSCD-1 leads to a desaturation of saturated fatty acids and facilitates their esterification and storage, thereby preventing downstream effects of lipotoxicity in HAECs. These findings add a novel aspect to the atheroprotective actions of LXR activators in cardiovascular disease.

liver X receptor activator; inflammation; interleukin-6; interleukin-8

THE METABOLIC SYNDROME WITH INSULIN RESISTANCE is a well-established major risk factor for cardiovascular disease (18, 35, 37, 38). A part of this increased cardiovascular risk is thought to be due to endothelial lipotoxicity, which is caused by elevated levels of circulating nonesterified free fatty acids (NEFAs), which are closely associated with insulin resistance (25, 31, 45). Lipotoxicity has been implicated in the pathogenesis of endothelial dysfunction with initiation of vascular inflammation, progression of atherosclerotic lesions, and, finally, atherosclerotic complications like atherothrombosis, stroke, and myocardial infarction. Endothelial dysfunction, determined by impaired endothelium-dependent vasodilatation, is considered to be an early functional stage of atherosclerosis accompanied by vascular inflammation that precedes morphological changes of the vessel wall (4) and is predictive for cardiovascular events (20, 41, 49). Elevated plasma NEFA levels are positively correlated with endothelial dysfunction (42). The acute effect of NEFAs on endothelial function in vivo has been studied by Steinberg et al. (48), who observed significant endothelial dysfunction in humans after elevation of circulating NEFA levels by lipid infusion. One widely proposed mechanism could be the induction of inflammatory cytokines in arterial endothelial cells (44). Distinct effects of saturated and unsaturated NEFAs have been observed, and apparently, the composition of free fatty acids is more relevant to endothelial function and vascular inflammation than the total amount of NEFAs (44, 46). The most abundant saturated fatty acid in human plasma, palmitate, has a high potency for the induction of inflammatory cytokines in endothelial cells, and the palmitate content of circulating NEFAs correlates with serum levels of IL-6 (44). These results provide a potential mechanism for how saturated NEFAs can trigger vascular inflammation and contribute to endothelial dysfunction in early stages of atherosclerosis. The role of NEFAs in the development of cardiovascular complications in later stages of atherosclerosis has also been attributed to the induction of endothelial cell apoptosis (13, 15, 45). Saturated NEFAs can induce endothelial cell apoptosis (45), leading to denudation of the endothelial layer and subsequent vessel thrombosis (15). This may be a critical mechanism for plaque erosion and atherothrombosis (13) and a cause of myocardial infarction or stroke. Elevated levels of NEFAs have been demonstrated to influence mortality in patients with clinically overt atherosclerosis (34). Both lipotoxic effects, inflammation and apoptosis, are induced by saturated NEFAs and can be reversed by addition of unsaturated NEFAs (30, 44, 45). Stearoyl-CoA desaturase is the limiting enzyme that converts saturated fatty acids to monounsaturated fatty acids, thus providing an endogenous source of unsaturated fatty acids. Therefore, enhancing the activity of human stearoyl-CoA desaturase-1 (hSCD-1) could be a strategy to prevent the toxic effects of saturated NEFAs.

A new group of substances activating the liver X receptor (LXR) has proven to possess antiatherogenic properties in atherosclerosis-prone mouse models apoE−/− and LDL receptor−/− (27, 50). The effect has been attributed mainly to an influence on reverse cholesterol transport and inhibition of inflammation. Through induction of the monocytc ATP-binding cassette transporter A1, excess cholesterol is transferred from atherosclerotic plaques to HDL and raises plasma HDL cholesterol levels. LXR activators have been shown to inhibit NF-κB-dependent induction of inflammatory gene expression in macrophages through not fully understood mechanisms (57).
However, in addition to genes linked to the reverse cholesterol transport, LXR agonists upregulate hepatic genes involved in fatty acid and triglyceride synthesis; among these is hSCD-1 (9, 26). In this study, we test the hypothesis that the LXR activator TO-901317 induces endothelial hSCD-1 expression, thereby providing a strategy to prevent lipotoxic effects of palmitate on endothelial cell viability and inflammation.

EXPERIMENTAL PROCEDURES

Cell culture. Human arterial endothelial cells (HAECs) were isolated from adult human iliac arteries of transplant donors by mechanically removing the endothelial layer as previously described (3) and were cultured in endothelial growth medium-2 (Clonetics, Walkersville, MD) containing 2% FCS on culture flasks (Greiner, Frickenhausen, Germany) coated with 2% gelatin. Immunocytochemical staining showing the human arterial endothelial cells (HAECs) express human stearoyl-CoA desaturase (hSCD-1) protein. The hSCD-1 antibody was tested in baby hamster kidney (BHK) cells transfected with hemagglutinin (HA)-tagged human SCD-1 and empty expression vector pcDNA3 (con). Endogenous SCD-1 in BHK cells is not detected by the human hSCD-1 antibody. Corresponding bands are recognized by the HA antibody (αHA) and the hSCD-1 antibody (αhSCD-1). Endogenous hSCD-1 expression in untransfected HepG2 liver cells, human myotubes, and HAECs was detected with the hSCD-1 antibody. B: palmitate does not induce hSCD-1 mRNA expression in HAECs. The influence of palmitate on hSCD-1 mRNA expression HAECs (B), coronary artery smooth muscle cells (CASMCs; C), primary human myotubes (hMT; D), and HepG2 hepatoma cells (E) is shown. Cells were incubated with palmitate 0.5 mM for 20 h and hSCD-1 mRNA content determined by RT-PCR. No significant induction is observed in HAECs (n = 7, P = 0.58). Palmitate induces hSCD-1 expression in myotubes (n = 40, P < 0.00001), HepG2 cells (n = 7, P < 0.001), and CASMCs (n = 7, P = 0.0003); ***P < 0.001; ****P < 0.00001.
significantly reduces the palmitate-induced cytotoxicity (5%, $P < 0.01$). hSCD-1 overexpression significantly reduces the palmitate-induced cytotoxicity (5%, $P < 0.05$).

**Transfection:**

A: hSCD-1 was overexpressed in human embryonic kidney (HEK)-293 cells by transient transfection. Immunoblotting against hSCD-1 shows a strong overexpression of hSCD-1 compared with endogenous hSCD-1 expression. The cytosolic glutamine:fructose-6-phosphate amidotransferase (GFAT) protein expression was used as a loading control. B: after 24 h, the cells were exposed to 0.6 mM palmitate or BSA for 36 h, and cytotoxicity was determined by lactate dehydrogenase (LDH) release into the medium. Triton X-100-lysed cells are set as 100% cytotoxicity. Palmitate is cytotoxic in control transfected cells (19%, $P < 0.01$). hSCD-1 overexpression significantly reduces the palmitate-induced cytotoxicity (5%, $P < 0.05$).

**Cell cycle analysis.** Confluent HAEcs were treated as indicated. Detached cells were harvested from the supernatant by centrifugation and added to the adherent cells harvested by trypsinization. Cells were washed with PBS, fixed in 70% ice-cold ethanol, centrifuged, and washed again with PBS. After being stained with propidium iodide, cells were subjected to flow cytometric analysis of DNA content using a Becton Dickinson (Heidelberg, Germany) FACScalibur cytometer. Percentages of cells in the different cell cycle phases were calculated by CellQuest software (Becton Dickinson).

**Determination of cytotoxicity.** After a preincubation with TO-901317 or DMSO, confluent cells were incubated with 0.6 mmol/l amide (10%) gel electrophoresis (55). Proteins were transferred to a nitrocellulose membrane by semidry electroblocting, and immunodetection was performed using a mouse monoclonal antibody against α-HA (Babco, Richmond, CA), full-length recombinant hSCD-1 (ab19862; Abcam, Cambridge, UK), and glutamine:fructose-6-phosphate amidotransferase (GFAT) (56).

**RT-PCR.** RNA isolation was performed using RNaseasy silica gel columns (Qiagen, Hilden, Germany). Total RNA treated with RNase-free DNase I was transcribed into cDNA using Avian myoblastosis virus reverse transcriptase and the first-strand cDNA kit from Roche Diagnostics. Quantitative PCR was performed with SYBR Green I dye on a LightCycler 2.0 according to the instructions of the manufacturer (Roche Diagnostics). The primers were obtained from Invitrogen (Karlsruhe, Germany). Measurements were performed in duplicate.

**Conditions for RT-PCR quantification of mRNAs were as follows:**

- hSCD-1: 5'-TGAGAGAGATATCTTCAGC-3', 5'-ACGAGAGCCTCTTCCTAT-3', annealing at 58°C, 45 cycles, 3 mmol/l MgCl2; IL-8: 5'-AAGAGACACCGGAGAAC-3', 5'-ACTTCTCCACCAACCTCTTCG-3', annealing at 68°C, 45 cycles, 3 mmol/l MgCl2; IL-6: 5'-CCAGCTATGAACCTCTTCTC-3', 5'-GCTTGTTCCTCACTACTCTC-3', annealing at 63°C, 45 cycles, 3 mmol/l MgCl2; GAPDH: 5'-GTTCTCTTCTTCTCTTGTCC-3', 5'-ACGACACCAACTTCCACCC-3', annealing at 67°C, 45 cycles, 4 mmol/l MgCl2; β-actin: 5'-GACGAAGAGGAGGATCTCTCA-3', 5'-AGCTGGATAGCAACGTACA-3', annealing at 67°C, 45 cycles, 4 mmol/l MgCl2; LXRα and LXRβ RT-PCR was performed in using a QuantiTect SYBR Green PCR kit and Quantitect Primer Assays (Hs_NR1H2_1_SG and Hs_NR1H3_1_SG) according to the manufacturer’s instructions (Qiagen).

**Fig. 2.** Overexpression of hSCD-1 reduces palmitate-induced cytotoxicity. A: hSCD-1 was overexpressed in human embryonic kidney (HEK)-293 cells by transient transfection. Immunoblotting against hSCD-1 shows a strong overexpression of hSCD-1 compared with endogenous hSCD-1 expression. The cytosolic glutamine:fructose-6-phosphate amidotransferase (GFAT) protein expression was used as a loading control. B: after 24 h, the cells were exposed to 0.6 mM palmitate or BSA for 36 h, and cytotoxicity was determined by lactate dehydrogenase (LDH) release into the medium. Triton X-100-lysed cells are set as 100% cytotoxicity. Palmitate is cytotoxic in control transfected cells (19%, $P < 0.01$). hSCD-1 overexpression significantly reduces the palmitate-induced cytotoxicity (5%, $P < 0.05$).

**Fig. 3.** HAECs express liver X receptor (LXRα and LXRβ). The mRNA of LXRα and LXRβ was detected by quantitative RT-PCR from HAECs, primary human myotubes, primary human hepatocytes, and HepG2 cells ($n = 4$). LXRα is the predominant isoform in HAECs and hepatocytes, whereas primary human myotubes express more of the LXRβ isoform.
hSCD-1 AND ENDOTHELIAL LIPOTOXICITY

RESULTS

SCD-1 protein is expressed in HAECs. hSCD-1 is a lipogenic enzyme previously shown to be expressed in tissues involved in fatty acid metabolism like liver, adipose tissue, skeletal muscle, and sebaceous glands (10, 23, 32, 40, 58, 59). In preliminary studies we observed significant mRNA expression of hSCD-1 in human vascular endothelial cells and smooth muscle cells. To study the protein expression of hSCD-1, the specificity of three commercially available antibodies against hSCD-1 was evaluated in BHK cells overexpressing recombinant human HA-tagged hSCD-1 (Fig. 1A). The HA tag was used to identify overexpressed hSCD-1 protein. No hSCD-1 signal could be detected in untransfected BHK cells. Only one antibody (Abcam mouse monoclonal anti-human SCD-1) recognized the recombinant as well as endogenous hSCD-1 in the untransfected hepatoma cell line HepG2 in primary human myotubes and in human arterial endothelial cells, whereas the other commercial antibodies (rabbit polyclonal anti-human SCD-1 and Abcam rabbit polyclonal anti-human SCD-1; Alpha Diagnostic) did not detect hSCD-1 in our system (data not shown). Separated on a 10% polyacrylamide gel, hSCD-1 appeared as a double band at 37 and 35 kDa, probably due to partial proteolysis at the NH₂ terminus, as described previously (22). The results indicate that hSCD-1 protein is expressed in HAEC.

The saturated fatty acid palmitate does not induce hSCD-1 mRNA expression in HAECs. The most abundant saturated NEFA in human plasma, palmitate, as a substrate of SCD-1 is involved in fatty acid metabolism like liver, adipose tissue, skeletal muscle, and sebaceous glands (10, 23, 32, 40, 58, 59). We studied whether palmitate can induce hSCD-1 expression in human cells. Using previously established experimental conditions (43, 44), palmitate (0.5 mM for 20 h) significantly induced expression of hSCD-1 mRNA in primary human myotubes (2.03 ± 0.16, P < 0.00001), primary human CASMCs (2.24 ± 0.22, P < 0.001), and HepG2 hepatoma cells (1.72 ± 0.13, P < 0.001) (Fig. 1, A–E). In HAECs, however, no increase in hSCD-1 expression was detectable (Fig. 1B). Accordingly, human coronary artery endothelial cells showed no significant increase in hSCD-1 mRNA (data not shown). Noteworthy is that primary human arterial endothelial cells are especially susceptible to saturated NEFA-induced lipoapoptosis compared with other cell types (45). This led us to the hypothesis that the high degree of lipoapoptosis observed in endothelial cells might be due to less pronounced hSCD-1 levels under saturated NEFA exposure.

Overexpression hSCD-1 reduces palmitate-induced cytotoxicity. To investigate the effect of SCD-1 on palmitate-induced cytotoxicity, we first used a transient overexpression system in HEK-293 cells (Fig. 2A). Cytotoxicity was determined as LDH

![Graph](http://ajpendo.physiology.org/DownloadedFrom/10.221012.335onOctober14,2017)
release into the culture medium. Palmitate was cytotoxic in control vector transfected HEK-293 cells (19 ± 4%, P < 0.01; untreated cells are set as 0% and lysed cells as 100%). The palmitate-induced cytotoxicity was abolished in hSCD-1-overexpressing cells, demonstrating a protective role of hSCD-1 (5 ± 0.4%, P < 0.05; Fig. 2B).

**LXRα and LXRβ are expressed in HAECs.** To establish a pharmacological model with increased hSCD-1 expression in HAECs, we treated cells with the LXR-activating compound TO-901317 for 20 h. Activators of LXR, apart from other effects, have been shown to induce SCD-1 expression in several tissues [e.g., in liver, skeletal muscle, kidney, and macrophages (9, 11, 52, 59)]. First, we investigated whether the LXR is expressed in HAECs. Two receptor isoforms, LXRα and LXRβ, occurring with tissue-specific distribution have been described (57). In a quantitative RT-PCR, we detected a strong mRNA expression of both isoforms LXRα and LXRβ in HAECs (Fig. 3). The predominant form in HAECs was LXRα with expression levels even higher than in HepG2 cells and primary human hepatocytes. In primary human myotubes, LXRβ was the predominant isoform.

**The LXR activator TO-901317 induces hSCD-1 expression in HAECs.** Next, we studied the effect of LXR activation on endothelial hSCD-1 expression. HAECs were treated with TO-901317 for 20 h, and the expression of hSCD-1 mRNA was measured by quantitative RT-PCR. Treatment with 10 μM TO-901317 increased the hSCD-1 mRNA in HAECs 3.0-fold (Fig. 4A). Treatment with TO-901317 at concentrations of 0.01–10 μM for 20 h increased hSCD-1 protein expression in a dose-dependent manner (Fig. 4, B and C). For further studies we used 10 μM to achieve a maximal LXR activation, as published previously (24). These data indicate that LXR activators stimulate hSCD-1 mRNA and protein expression in human endothelial cells.

**The LXR activator TO-901317 increases hSCD-1 activity in HAECs.** To evaluate whether TO-901317 treatment-induced increase in hSCD-1 expression leads to enhanced desaturation of palmitate and stearate, the composition of cellular fatty acids was analyzed. HAECs were also exposed to palmitate for 5 h to observe the effect under conditions of substrate excess (Table 1). Under basal conditions, exposure to TO-901317 led to a significant increase of the C16:1 content and the SCD-1 activity index, estimated from C16:1/C16:0 ratio. When exposed to palmitate for 5 h, the SCD-1 activity index for both C16:1/C16:0 and C18:1/C18:0 was elevated by TO-901317. These data indicate that increased SCD-1 mRNA and protein levels by treatment with TO-901317 result in increased enzyme activity, estimated from product/precursor ratios in HAECs. Therefore, treatment with TO-901317 is useful to study effects of pharmacological induction of hSCD-1 in HAECs.

**The LXR activator TO-901317 reduces palmitate-induced cytotoxicity and apoptosis in HAECs.** We hypothesize that induction of hSCD-1 expression and activity by TO-901317 prior to exposure to palmitate can reduce lipotoxicity in HAECs by desaturating and thereby detoxifying palmitate. Since preliminary experiments showed that cytotoxicity and apoptosis are induced after treatment with 0.6 mM palmitate for 36 h, this experimental condition was used. Exposure to palmitate caused 41 ± 4% cytotoxicity in HAECs, as determined by an LDH release cytotoxicity assay (P < 0.00001; data not shown). Preincubation of HAECs with the LXR activator TO-901317 significantly reduced the palmitate-induced cytotoxicity by 27% (Fig. 5A). However, induction of hSCD-1 expression is only one of many effects of LXR activators (9, 26). To prove the hypothesis that this effect of TO-901317 is due to increased hSCD-1 expression and enzymatic activity, we first used a pharmacological approach in HAECs (7, 8). We applied 10/12 CLA, an inhibitor of hSCD-1 activity (8, 33), and the chemically related but inactive analog 9/11 CLA as control substances (Fig. 5A). Inhibition of hSCD-1 by 10 μM 10/12 CLA reversed the protective effect of TO-901317 against lipotoxicity. The chemically related but inactive 9/11 CLA (7, 8, 33) had no effect on TO-901317-induced protection from lipotoxicity. Both 10/12 CLA and 9/11 CLA alone had no effect on cell viability under these conditions (data not shown).

Next, we investigated the SCD-1 activity indexes upon treatment with TO-901317 and in addition to 10/12 CLA and 9/11 CLA (Fig. 5B). The cells were treated with TO-901317 for 20 h, followed by palmitate 0.5 mM for 5 h. Ten micromolars CLA was added 30 min prior to the treatments. TO-901317 significantly increased SCD-1 activity indexes. This increase was inhibited to control levels by the SCD-1 inhibitor 10/12 CLA. The inactive control substance 9/11 CLA did not reduce TO-901317-stimulated SCD-1 activity indexes. Activity indexes with TO-901317 plus 9/11 CLA remained significantly higher than control or TO-901317 plus 10/12 CLA-treated cells (Fig. 5B). Incubation with 10 μM 10/12 CLA for 20 h did not significantly influence hSCD-1 mRNA expression in HAECs (93 ± 20% of control, P = 0.72, n = 6, normalized to GAPDH). These data indicate that 10 μM 10/12 CLA inhibits hSCD-1 activity in HAECs under the used experimental conditions.

To confirm that TO-901317 not only reduces palmitate-induced cytotoxicity but also prevents the initiation of apoptosis in HAECs, we measured the proportion of cells with a subG1 DNA content in a cell cycle assay. Palmitate induced

| Table 1. Effect of TO-901317 on fatty acid distribution and desaturation of fatty acids in HAECs under basal and palmitate-stimulated conditions |
|-------------------------------|-----------------|-----------------|-----------------|------------------|------------------|
|                               | Basal           | Palmitate       |                 |                  |                  |
|                               | DMSO            | +TO-901317      | P               | DMSO             | +TO-901317       |
| SCD-1 index ([16:1/16:0] × 100)| 9.6 ± 1.3       | 14.3 ± 1.6      | 0.003           | 6.0 ± 1.1        | 10.4 ± 2.4       | 0.005           |
| Palmitate (C16:0)             | 30.0 ± 1.5%     | 30.2 ± 0.8%     | NS              | 43.0 ± 2.2%      | 43.1 ± 3.4%      | NS              |
| Palmitoleate (C16:1)          | 2.9 ± 0.2%      | 4.3 ± 0.4%      | 0.005           | 2.6 ± 0.4%       | 4.4 ± 0.4%       | 0.001           |
| SCD-1 index ([18:1/18:0] × 100)| 88.7 ± 4.1      | 98.0 ± 2.6      | 0.082           | 78.7 ± 4.3       | 88.0 ± 5.4       | 0.016           |
| Stearate (C18:0)              | 20.0 ± 0.3%     | 19.2 ± 0.2%     | NS              | 17.0 ± 0.8%      | 16.3 ± 0.4%      | NS              |
| Oleate (C18:1N9)              | 17.8 ± 0.9%     | 19.5 ± 0.9%     | NS              | 13.6 ± 1.4%      | 14.3 ± 1.1%      | NS              |

Values are means ± SE. HAECs, human arterial endothelial cells; SCD-1, stearoyl-CoA desaturase-1; NS, not significant. The fatty acid distribution is stated in %total cellular fatty acid content.
apoptosis in HAECs (Fig. 5C). The apoptosis rate was significantly reduced by preincubation with the LXR activator TO-901317 (66 ± 7%, P = 0.002; Fig. 5D). Corresponding to the results of the cytotoxicity assays, the protective effects of TO-901317 were reversed by the hSCD-1 inhibitor 10/12 CLA (100 ± 14%). Thus, the induction of hSCD-1 is necessary for the protective effects of TO-901317 against lipoapoptosis.

SCD-1 knockdown prevents reduction of palmitate-induced cytotoxicity by TO-901317. To further confirm relevance of hSCD-1 for the protective effects of TO-901317 against lipotoxicity on a molecular level, we reduced hSCD-1 protein expression by siRNA knockdown in HEK-293 cells (Fig. 6A). In control transfected HEK-293 cells, TO-901317 reduced palmitate-induced cytotoxicity by 58%. This effect was almost abolished by
knockdown of hSCD-1 (Fig. 6B). These results together with the results shown in Fig. 5 provide molecular and pharmacological evidence that the induction of hSCD-1 expression and hSCD-1 enzyme activity is necessary for the protective effects of the LXR activator TO-901317 against lipotoxicity.

The LXR activator TO-901317 prevents palmitate-induced expression of inflammatory cytokines IL-6 and IL-8 in HAECs.

An important aspect of lipotoxicity in endothelial cells is the induction of inflammatory cytokines and triggering of local inflammation in the vessel wall. Exposure to palmitate (0.5 mM for 20 h) led to a strong induction of IL-6 mRNA expression (29.2 ± 7.0-fold, P = 0.02) (Fig. 7A). In previous studies, these experimental conditions also increased IL-6 protein production (44). Of note, no apoptosis under this shorter exposure to palmitate was observed other than that used for cytotoxicity assays (20 h 0.5 mM) (44). Preincubation with 10 μM TO-901317 significantly reduced this IL-6 induction by 63% (10.8 ± 1.8-fold, P = 0.01). Similarly, the expression of IL-8 was induced by palmitate (15.2 ± 4.3-fold, P = 0.05), with a significant reduction after pretreatment with 10 μM TO-901317 by 60% (6.3 ± 1.0-fold, P < 0.01) (Fig. 7B). Addition of the SCD-1 inhibitor 10/12 CLA attenuated the protective effect of TO-901317 on IL-6 expression (P = 0.03) and abolished the effect on IL-8 induction (P < 0.01), whereas the effect of palmitate alone remained largely unaltered (Fig. 7, A and B). These results indicate that reduction of palmitate-induced cytokine expression by TO-901317 is dependent on induction of SCD-1.

Induction of hSCD-1 facilitates lipid accumulation in saturated NEFA-exposed HAECs. To demonstrate that the induction of hSCD-1 by TO-901317 leads to enhanced storage of exogenous saturated fatty acids in triglycerides, we performed lipid staining in endothelial cells. In the absence of free fatty acids, HAECs did not contain any lipid droplets detectable in an Oil Red O staining. Upon treatment with unsaturated NEFAs like 0.6 mM oleate for 2 h, a massive accumulation of small perinuclear lipid droplets became visible (Fig. 8A). Using palmitate or stearate, no lipid accumulation in HAECs was found. After preincubation with the LXR activator TO-901317 (10 μM for 20 h), formation of lipid droplets became visible upon palmitate and stearate exposure, comparable with cells exposed to oleate. To confirm these observations, triglycerides were measured in whole cell lysates of endothelial cells incubated with palmitate or stearate. 

Fig. 6. A: as a 2nd method, a small interfering RNA (siRNA) knockdown of hSCD-1 was generated in HEK-293 cells. Immunoblotting against hSCD-1 shows an almost complete knockdown of hSCD-1. GFAT is used as a loading control. B: the reduction of palmitate-induced cytotoxicity by TO-901317 observed in control transfected HEK-293 cells (~58% P < 0.01) was inhibited in hSCD-1 knockdown cells (~29% P = 0.26). After treatment with TO-901317, SCD-1 knockdown cells showed a higher cytotoxicity than control transfected cells [42 (con) vs. 93% (SCD-1), P < 0.01]. **P < 0.01.

Fig. 7. The LXR activator TO-901317 reduces palmitate-induced mRNA induction of inflammatory cytokines IL-6 (A) and IL-8 (B) in HAECs. Inhibition of SCD-1 with 10/12 CLA reverses this effect. Cells were incubated with 0.5 mM palmitate for 20 h with or without preincubation with 10 μM TO-901317. Reduction with TO-901317: IL-6 P = 0.01, IL-8 P < 0.01; reduction with TO-901317 palmitate vs. palmitate with 10/12 CLA: IL-6 P = 0.03, IL-8 P < 0.01. *P < 0.05; **P < 0.01.
for 5 h with palmitate or oleate (Fig. 8B). Oleate led to a significant increase in triglyceride content. Palmitate was stored only as triglycerides after preincubation with TO-901317, but not under basal conditions. Together, the results show a rapid and unexpected lipid accumulation in HAECs after exposure to NEFA but only when unsaturated NEFAs are provided either extracellularly or by SCD-1-mediated desaturation of NEFAs.

**DISCUSSION**

Disadvantageous effects of saturated fatty acids on endothelial function are well described in vivo and in vitro. Although saturated fatty acids palmitate and stearate cannot be stored and induce apoptosis in primary human arterial endothelial cells, unsaturated fatty acids like oleate or palmitoleate are not toxic and can be stored as triglycerides (30). Since the pathobiology underlying the particularly deleterious effects of saturated fatty acids in the vasculature are unclear, we set out to elucidate the molecular mechanism and preventative strategies in primary HAECs.

The major findings of our studies are that 1) HAECs express hSCD-1 protein, 2) the expression of hSCD-1 is not enhanced by palmitate treatment, whereas palmitate induces hSCD-1 expression in human myotubes, HepG2, and human coronary artery smooth muscle cells, 3) overexpression of hSCD-1 attenuates palmitate-induced toxicity, 4) the LXR activator TO-901317 induces hSCD-1 expression and activity in HAECs and reduces palmitate-induced cytotoxicity, apoptosis, and inflammatory cytokine expression, 5) these effects can be attributed to hSCD-1 activity since inhibition with 10/12 CLA, but not the inactive 9/11 CLA, attenuates the benign TO-901317 effects, 6) TO-901317 does not protect from lipotoxicity in an SCD-1 knockdown model, and 7) HAECs may rapidly (2–5 h) accumulate triglycerides upon treatment with oleate; saturated NEFAs led only to lipid accumulation when
cells were preincubated with TO-901317. Vascular endothelial cells display remarkable heterogeneity between species and even between veins and arteries of different vascular beds (2, 5, 28, 39). Because atherosclerotic lesions predominantly occur in large vessels first, we used large vessel-derived (1) primary human arterial endothelial cells from healthy individuals (3) throughout our studies to stay as close to the human situation as possible. Due to a limited transfection capacity of primary human cells, we used HEK-293 cells for some molecular studies. The NEFA concentrations used in the experiments are also within the range that is reached in the plasma of healthy human subjects (44). Our results suggest that the lipotoxic effects of saturated fatty acids on HAECs are caused by their inability to respond to this challenge by inducing the expression of hSCD-1 with subsequent conversion of the saturated to monounsaturated fatty acids, which in turn, together with saturated fatty acids, can be incorporated into neutral lipids. In Chinese hamster ovary cells, unsaturated fatty acids, either by exogenous addition or by endogenous desaturation through overexpression of SCD-1, rescue palmitate-induced apoptosis by channeling palmitate into triglyceride pools away from pathways leading to apoptosis (30). In the murine pancreatic β-cell line MIN6, the selection of a palmitate-resistant subpopulation that is characterized by increased SCD-1 expression and inducibility by palmitate was reported (7). Supporting these data, loss of SCD-1 worsens diabetes in leptin-deficient obese mice through accelerated β-cell failure (16). SCD-1 appears to be necessary for triglyceride storage, as knockout mouse models strike by a lack of hepatic triglycerides and prevention of hepatic steatosis in ob/ob mice (10). Therefore, we conclude that the LXR activator TO-901317 protects against palmitate-induced lipotoxicity by increased hSCD-1-mediated desaturation of palmitate to palmitoleate and incorporation into triglycerides. Accumulation of triglycerides in nondiopose tissues appears to be a measure of the lipid-overload state associated with insulin resistance, hyperlipidemia, and obesity. Our experiments, similarly to results obtained in Chinese hamster ovary and pancreatic β-cells, indicate that cellular lipid accumulation itself is not initially toxic (7,30). Rather, deposition of excess NEFA in lipid depots withdraws these metabolites from pathways to exerting their deleterious effects. Rapid storage in neutral lipids can be a mechanism of protecting the cells from postprandially occurring peak levels of circulating NEFAs. However, in chronic states of increased NEFAs when cellular capacity of lipid storage is exceeded, intracellular NEFA levels may increase and exert toxic effects. Together, these are several arguments for why SCD-1 expression is crucial to the prevention of lipotoxicity induced by saturated NEFAs.

The LXR activators like TO-901317 have proven antiatherogenic properties in mouse models (27,50). The effect has been attributed mainly to an influence on reverse cholesterol transport and inhibition of NF-κB activation in macrophages (19,27,57). We have recently demonstrated NF-κB-dependent induction of apoptosis by palmitate in endothelial cells (45). Our data show that the LXR activator TO-901317 additionally prevents initial steps of palmitate-induced lipotoxicity through induction of hSCD-1 since the observed protective effects were inhibited by SCD-1 knockdown or the SCD-1 inhibitor 10/12 CLA. The present study provides evidence that protection of the LXR activator TO-901317 against atherosclerosis can be, at least in part, due to reduced NEFA-induced endothelial cell apoptosis and inflammation. While this article was in preparation, lamarin shear stress-induced expression of SCD-1 in vascular endothelial cells was reported, adding further importance to our findings (36). Those authors postulated a contribution of endothelial SCD-1 expression to the antiatherosclerotic effects of laminar flow in straight vessels. Pharmacological prevention of lipotoxicity in endothelial cells represents a valuable tool for vascular protection reaching from initiation of endothelial dysfunction to vascular complications, myocardial infarction, and stroke. This adds a novel aspect to the protective mechanisms of LXR-activating drugs in vascular disease. Activation of hepatic SREBP-1c target genes and consecutive hepatic steatosis, as well as unfavorable plasma lipid profile with elevated plasma triglycerides, are seen as serious side effects of LXR activators as therapeutic agents that could limit their applicability (19). On the other hand, inhibition of hepatic SCD-1 activity is currently discussed as potential treatment for obesity, insulin resistance, and the metabolic syndrome (10,14,17,21). An unselective inhibition of SCD-1 that includes vascular endothelial cells is likely to increase lipotoxicity in these cells, thus augmenting atherosclerosis. Thus, this study stresses the potential opposing disease-related effects of SCD-1 in different tissues and demonstrates the need for tissue-specific acting drugs.

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