Laminar shear stress up-regulates the expression of stearoyl-CoA desaturase-1 in vascular endothelial cells

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Abstract

Objective: Laminar shear stress plays critical roles in vascular homeostasis and exerts various metabolic effects on endothelial cells (ECs). Stearoyl-CoA desaturase-1 (SCD1), which catalyzes the biosynthesis of monounsaturated fatty acids, affects the lipid composition and fluidity of the cell membrane. Thus, we examined the effect of laminar flow on SCD1 expression in ECs.

Methods: A flow chamber was used to impose a laminar shear stress on a confluent monolayer of human vascular ECs. The expression of SCD1 was examined using real-time RT-PCR and Northern and Western blotting. Immunohistochemical staining was used to assess the expression of SCD1 in Sprague-Dawley rat arteries, including the sites of arterial bifurcation.

Results: Laminar shear stress (12 dyn/cm², 12 h) markedly increased the gene expression of SCD1 in ECs. The flow-induced SCD1 expression was attenuated by peroxisome proliferator-activated receptor (PPAR)-γ antagonists both in vitro and in vivo. Troglitazone and rosiglitazone significantly increased the gene expression of SCD1. Furthermore, overexpression of a constitutively active PPARγ induced the expression of SCD1 in ECs. Immunohistochemical study of cross-sections from rat celiac arteries revealed that endothelial expression of SCD1 was substantially higher on the medial division apex, where the shear stress is high and more laminar, than the lateral aspect, where the shear stress is low and unsteady.

Conclusion: These in vitro and in vivo results demonstrate that laminar flow increased the expression of SCD1 in endothelium through a PPARγ-specific mechanism, which may contribute to the shear stress-mediated protective roles in ECs.

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1. Introduction

Vascular endothelium, being constantly exposed to hemodynamic forces, plays an important role in sensing the alterations in biological, chemical and physical properties in blood flow to maintain physiological homeostasis. Shear stress, the frictional force created by blood flow, exerts a variety of effects on endothelial structure and function, and contributes to the focal distribution of atherosclerotic lesions in the vessels. The straight parts of the arterial tree, generally spared from atherogenesis, are exposed to laminar shear stress with a large net forward component. In endothelial cells (ECs), steady laminar flow resulted in changes in cytoskeletal organization, modulation of molecular signaling, and inhibition of cell proliferation and apoptosis, whereas disturbed flow generally has little or even opposite
effects [1]. Recent genomic and proteomic studies indicate that shear stress may exert complex effects by eliciting distinct changes in gene expression profiles in ECs [2]. It has been demonstrated that shear stress can stimulate cell metabolism and increase the membrane fluidity of vascular endothelial cells [3,4]. The mechanisms that mediate shear stress induced alterations in membrane and lipid metabolism and fluidity remain to be elucidated.

The degree of fatty acid unsaturation in cell membrane lipids determines membrane fluidity, the alteration of which has been implicated in a variety of disease states, including diabetes, obesity, hypertension, cancer, and neurological and heart diseases [5–7]. Stearoyl-CoA desaturase1 (SCD1), anchored in the endoplasmic reticulum, is a rate-limiting desaturase that catalyzes the Δ9-cis desaturation of saturated fatty acids, converts palmitate and stearate into palmitoleate and oleate, the predominant unsaturated fatty acids present in membrane phospholipids [7,8]. Since altered SCD1 expression and activity could lead to changes in cell membrane phospholipid composition and fluidity, the regulation of SCD1 is of physiological importance. Accumulating evidence has demonstrated that shear stress has profound effects on cell lipid metabolism and membrane components. For instance, we have previously shown that shear stress activates peroxisome proliferator-activated receptor-γ (PPARγ) and sterol-responsive element binding protein-1 (SREBP-1), two lipogenic transcription regulators [9,10]. Shear stress also induces membrane translocation of caveolin-1 and formation of caveola microdomains, which may play important roles in mechanotransduction in ECs [11]. Here, we sought to examine whether laminar shear stress can regulate the gene expression of SCD1 in ECs in vitro and whether the SCD1 expression is related to hemodynamics in vivo.

2. Materials and methods

2.1. Cells and reagents

Human umbilical vein endothelial cells (HUVECs) were harvested by collagenase treatment of umbilical cord veins and cultured on plates coated with collagen. Cells were maintained in M199 supplemented with 20% fetal bovine serum, 20 mM HEPES (pH 7.4), 1 ng/ml of recombinant human fibroblast growth factor, and 90 μg/ml of heparin (Sigma-Aldrich, Louis, MO, USA) [12]. For all experiments, HUVECs within passage 3 were used. The investigation conforms with the principles outlined in the Declaration of Helsinki for use of human tissue. GW9662, troglitazone and rosiglitazone were purchased from Caymen Chemicals (Ann Arbor, MI, USA) and dissolved in dimethyl sulfoxide (DMSO).

2.2. Flow experiments

The flow experiments were performed as previously described [13,14]. A parallel-plate flow system was used to impose a laminar shear stress of 12 dyn/cm² or 2 dyn/cm² by perfusing the culture media over a confluent monolayer of ECs seeded on a glass slide on the bottom of the flow chamber. The flow system was kept at 37 °C and ventilated with 95% humidified air containing 5% CO₂.

2.3. Plasmids and adenoviral vectors

The PPRE-TK-Luc is a luciferase reporter containing the herpes virus thymidine kinase promoter (105/+51) downstream of three copies of PPAR-response elements (PPRE) from the acyl-CoA oxidase gene [15]. HUVECs were transfected with the use of a cationic lipid-based transfection reagent Targefect (Targeting System, San Diego, CA). At 48 h after transfection, cell lysates were harvested to measure luciferase activity. In all experiments, pRSV-β-gal was co-transfected, and the β-galactosidase activity was measured to normalize transfection efficiency. The pVP-PPARγ was a plasmid encoding the mouse PPARγ1 fused with a VP16 minimal transactivator domain to its N-terminus. The recombinant adenoviruses expressing VP-PPARγ (Ad-VP-PPARγ), green fluorescence protein (Ad-GFP) and tetracycline transactivator (Ad-tTA) were previously described [12,15]. The expression of PPARγ and GFP was under the control of a tet operator (7 × tet). The transgene expression was switched off in the presence of tetracycline in the culture medium. For adenosine-mediated gene transfer, confluent HUVECs were exposed to adenoviral vectors at a multiplicity of infection of 100 for 2 h (Ad-tTA was co-infected to induce the tetracycline controllable expression). After the viruses had been washed off, the infected ECs were further incubated for indicated periods for subsequent analyses.

2.4. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) and northern blotting

Total RNA was extracted with the use of TRIzol reagent (Invitrogen). Two μg of total RNA was converted into cDNA with the use of reverse transcriptase and oligo (dT) (Promega, Madison, WI, USA) as a primer. Real-time quantitative PCR was performed using the iQ™ SYBR Green PCR Supermix in the DNA Engine Opticon real-time system (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the results were analyzed with the Opticon Monitor analysis software. In each experiment, data were expressed as fold changes of the treatment groups in relation to the controls, which were set as 1. The sequences of the primers were as follows: SCD1: 5′-CTC TAC TGC TGG ACA TGA GA-3′ (forward), 5′-AAT GAG TGA AGG GGC ACA AC-3′ (reverse); β-actin: 5′-ATC TGG CAC CAC ACC TTC-3′ (forward) and 5′-AGC CAG GTC CAG ACG CA-3′ (reverse). Northern blotting was performed as previously described [12]. Briefly, 15 μg of total RNA was separated on a 1.0% agarose/formaldehyde gel, transferred onto a nylon membrane, and hybridized with cDNA probes for SCD1. The cDNA probes for human
SCD1 and β-actin were synthesized with the use of reverse-transcriptase PCR, purified, and labeled with [32P]dCTP with the use of the Prime-a-Gene® Labeling System (Promega, Carlsbad, CA, USA) according to the manufacturer’s instructions.

2.5. Western blotting

Cellular proteins were extracted from HUVECs as previously described [12,15]. Cell lysates were resolved by 12% SDS-PAGE for electrophoresis and transferred onto a nitrocellulose membrane (Pall Corp, NY). SCD1 protein was reacted with a goat anti-SCD1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The bound primary antibodies were recognized with the use of a horseradish peroxidase (HRP)-conjugated secondary antibody against goat IgG (Sigma) and visualized by the ECL detection system (Cell Signaling, Beverly, MA). Signal intensity was scanned, analyzed with NIH Image J and expressed as fold change in relation to the control.

2.6. Immunohistochemistry

Adult male Sprague–Dawley rats weighing 300–350 g were used for the in vivo studies. Investigations were conducted conforming to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publications No. 85-23, revised 1996). The experimental protocol was approved by the Institutional Committee for Laboratory Animal Care. PPARγ antagonist bisphenol A diglycidyl ether (BADGE) was administrated via intraperitoneal injection (10 mg/kg/day) for 7 days. The control rats were injected with the equal volume of phosphate-buffered saline (PBS). The rats were euthanized with 0.6% pentobarbital. The arterial system was washed free of blood by perfusing via the left ventricle with 100 ml of PBS at a pressure of 100 mmHg and fixed with 4% paraformaldehyde for immunohistochemical studies. Eight μm-sections were prepared from OCT-embedded arteries of Sprague-Dawley rats. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 15 min. The sections were reacted with a goat-anti-SCD1 antibody or rabbit anti-von Willebrand factor (vWF) antibody (Zymed, San Francisco, CA, USA). After washing with PBS, the sections were incubated with HRP-conjugated secondary antibody against goat or rabbit IgG (Sigma). Diaminobenzidine tetrahydrochloride (DAB) was used for the color reaction. The results were examined by light microscopy, and images were acquired by using a digital camera. Negative controls were performed for all immunohistochemical staining with the use of species- and isotype-matched IgG [14].

2.7. Statistical analysis

The data are expressed as means±SEM. Statistical analyses were performed with one-way ANOVA followed by the Student–Newman–Keuls test or Student’s t-test, with statistical significance set at P<0.05.

3. Results

3.1. Laminar flow up-regulates SCD1 gene expression in ECs

To examine the effect of laminar flow on SCD1 gene expression, we applied a laminar flow with a physiological level of shear stress at 12 dyn/cm² or a low shear stress Fig. 1. Laminar flow-induced SCD1 mRNA expression in ECs. (A) ECs were exposed to laminar flow (12 dyn/cm²), low shear (2 dyn/cm²) or kept under a static condition for 12 h. SCD1 gene expression was analyzed with qRT-PCR. The levels of SCD1 mRNA were normalized to the β-actin level and expressed as fold of increase in relation to the control. The data represent means±SEM of 3 independent experiments. **P<0.01 vs. control. (B) ECs were exposed to laminar flow (12 dyn/cm²) for 0, 3, 6, 12 or 24 h. Northern blots were hybridized with [32P]-labeled cDNA probes for human SCD1 and β-actin.

**Fig. 2. PPARγ antagonist GW9662 inhibited the laminar flow-induced SCD1 gene expression. HUVECs were pretreated with GW9662 (10 μM for 30 min) or control (DMSO 1:1000) before the exposure to laminar flow or static condition. The SCD1 mRNA expression was analyzed with the use of real-time RT-PCR. The data represent the means±SEM of 3 independent experiments. **P<0.01 vs. DMSO.
(2 dyn/cm²) to confluent HUVECs for 12 h. The mRNA level of SCD1 was determined by real-time RT-PCR. As shown in Fig. 1A, laminar flow at 12 dyn/cm², but not a low shear stress (2 dyn/cm²), increased the expression of SCD1 mRNA (3.2±0.3 folds of static control, \( P<0.01 \)). The effect of laminar shear stress (12 dyn/cm²) on SCD1 gene expression was further examined at different time points. As confirmed by northern blot analysis (Fig. 1B), the induction of SCD1 mRNA transcript (5.2 kb) appeared at 6 h, reached the peak at 12 h and remained 24 h after the exposure to shear stress. These results demonstrated that laminar shear stress increased the SCD1 expression in ECs in a both intensity- and time-dependent manner.

3.2. Laminar flow induces SCD1 gene expression in a PPARγ-dependent manner

Since laminar flow was shown to activate PPARγ in ECs in a ligand-dependent manner [10,16], to assess whether PPARγ was responsible for the laminar flow-enhanced gene expression of SCD1 in ECs, we pretreated the cells with GW9662 (10 \( \mu \)M, 30 min), a PPARγ-specific antagonist, before exposure to laminar flow. As shown in Fig. 2, GW9662 did not affect the SCD1 expression under static condition but significantly attenuated the flow-induced SCD1 expression, indicating the contribution of PPARγ in the shear-induced gene expression of SCD1.

Fig. 3. Troglitazone and rosiglitazone increased the expression of SCD1 in ECs. (A) HUVECs were treated with troglitazone (50 \( \mu \)M) or rosiglitazone (10 \( \mu \)M) for 0, 6, 12 and 24 h. (B) HUVECs were treated with various concentrations of troglitazone or rosiglitazone for 24 h. Total RNA was isolated and examined with real-time RT-PCR for SCD1 mRNA expression. (C) HUVECs were treated with troglitazone or rosiglitazone at the indicated concentrations for 24 h. Western blotting was performed with primary antibodies against SCD1 or tubulin. Data represent mean±SEM of 3 independent experiments and are expressed as fold of increase compared to controls. * \( P<0.05 \), ** \( P<0.01 \) vs. control.
3.3. Effect of PPARγ agonists on the expression of SCD1 in endothelial cells

Since fluid shear stress can induce the production of PPARγ ligands [10,16], we next examined the effects of PPARγ ligand, the thiazolidinediones (TZDs) troglitazone and rosiglitazone, on SCD1 mRNA expression in ECs. As shown in Fig. 3A and B, troglitazone and rosiglitazone both significantly enhanced SCD1 mRNA expression in a time- and dose-dependent fashion. To further examine whether troglitazone and rosiglitazone regulate the expression of SCD1 at the protein level, Western blot analyses were performed. As shown in Fig. 3C, the amount of SCD1 protein was significantly increased in ECs incubated with troglitazone (10 to 100 μM) or rosiglitazone (1 to 10 μM) for 24 h, consistent with the increases at mRNA levels. Since some effects of PPARγ agonists were reported as receptor-independent, we pretreated ECs with GW9662 (10 μM, for 30 min) before exposure to rosiglitazone (Rosi) for 24 h. Total RNA was extracted and examined for SCD1 mRNA expression with the use of real-time RT-PCR. (B) GW9662 blocked rosiglitazone-induced SCD protein expression. Data represent mean±SEM of 3 independent experiments. **P<0.01.

3.4. Constitutive activation of PPARγ induces the expression of SCD1 in ECs

A constitutively active PPARγ (VP-PPARγ) was used to determine the effect of PPARγ on the gene expression of SCD1. As shown in Fig. 5A, overexpression of a plasmid encoding the VP-PPARγ induced a constitutive activation of PPRE-driven reporter gene without exogenous ligands. In further experiments, HUVECs were infected with the adenoviruses expressing the VP-PPARγ and tTA and

![Fig. 4. PPARγ antagonist blocked TZD-induced SCD1 expression in ECs. (A) HUVECs were pretreated with GW9662 (10 μM) or solvent for 30 min before exposure to rosiglitazone (Rosi) for 24 h. Total RNA was extracted and examined for SCD1 mRNA expression with the use of real-time RT-PCR. (B) GW9662 blocked rosiglitazone-induced SCD protein expression. Data represent mean±SEM of 3 independent experiments. **P<0.01.](image)

![Fig. 5. Constitutive activation of PPARγ induced the expression of SCD1 in ECs. (A) VP-PPARγ induced the expression of SCD1. (B) VP-PPARγ induced the expression of SCD1 mRNA. (C) VP-PPARγ induced the expression of SCD1 protein. Data represent mean±SEM of 3 independent experiments. *P<0.05, **P<0.01.](image)
maintained in the presence or absence of tetracycline. Supplement with tetracycline (0.1 μg/mL) completely suppressed the transgene expression and thus served as a control for the viral vector. Real-time RT-PCR revealed that constitutive activation of PPARγ significantly induced the mRNA expression of SCD1 (Fig. 5B), whereas overexpression of GFP had no effect. VP-PPARγ also induced the expression of SCD1 at protein level (Fig. 5C). These results indicated that PPARγ activation was sufficient to upregulate the expression of SCD1 in ECs.

3.5. Expression of SCD1 in ECs is related to hemodynamics in rat arteries

The up-regulation of SCD1 expression found in cultured ECs by laminar flow in vitro prompted us to examine whether such a flow-regulated SCD1 expression may also exist in arteries in vivo. Therefore, the branching points between the abdominal aorta and the celiac artery of normal rats were investigated with the use of immunohistochemistry. Sections around the branching point were

![Immunohistochemical detection of SCD1 in rat arteries. (A) Cross-section of rat celiac artery branch points, including the abdominal aorta and celiac artery, immunohistochemically stained with anti-SCD1. Enlarged views are shown for areas in the abdominal aorta (a) and the medial (b) and lateral (c) aspects of the celiac artery. Dotted line in drawing shows the plane at which the vessels were sectioned. (B) Antibody against von Willebrand factor (vWF) was used to confirm the endothelial integrity throughout the lumen areas. The sections were counterstained with hematoxylin. The arrows indicate the endothelium of the vessel. (C) SCD1 expression in abdominal aorta (a) and the medial (b) and lateral (c) aspects of the celiac arteries from rats treated with BADGE (lower panel) or control (upper panel) for 7 days. The results shown are representative of 3 animals in each group.](image-url)
immunostained with an anti-SCD1 antibody (Fig. 6A). In the abdominal aorta, SCD1 expression was continuous throughout all ECs (Fig. 6A-a). Notably, the highest expression was detected in adventitia, which consists of ample amounts of adipose tissues. The negative controls performed by replacing the primary antibody with an isotype-matched IgG did not result in detectable staining (data not shown). In the celiac artery, substantial differences in SCD1 expression were observed between its medial and lateral aspects. The expression level of SCD1 on the medial division apex (Fig. 6A-b), where the shear stress is high and more laminar, was much higher than that on the lateral aspects (Fig. 6A-c), where secondary flow and flow separation are more likely to occur. Thus, the anatomically related differences in SCD1 expression may be associated with the regional hemodynamic properties. Immunohistochemical staining with the use of an antibody against vWF confirmed the integrity of ECs (Fig. 6B). As shown in Fig. 6C, treatment with PPARγ antagonist BADGE markedly decreased endothelial expression of SCD1 in rat abdominal aorta and in celiac arteries on both lateral and medial aspects, indicating that the flow-associated endothelial expression of SCD1 is PPARγ-dependent.

4. Discussion

In this study, we present a novel finding that laminar flow increases the expression of SCD1 in endothelial cells via a PPARγ-dependent pathway. We also demonstrate that the SCD1 expression in the rat arterial endothelium is related to flow dynamics in vivo.

SCD is an iron-containing enzyme that catalyzes a rate-limiting step in the synthesis of unsaturated fatty acids. The principal product of SCD is oleic acid, which is formed by desaturation of stearic acid. The ratio of stearic acid to oleic acid has been implicated in the regulation of cell growth and differentiation through effects on membrane fluidity and signal transduction [7]. Under normal dietary conditions, SCD1 mRNA is highly expressed in adipose tissue and sebaceous glands. In vitro and in vivo evidence in the present study showed that SCD1 is actively expressed in ECs at both mRNA and protein levels. This is consistent with results of an earlier study showing Δ9-desaturase activity in cultured HUVECs [17]. In addition, the immunohistochemical results showed that the SCD1 expression is at a higher level in endothelium compared to that in smooth muscle cells, indicating a high rate of fatty acid desaturation in ECs. Notably, the strongest SCD1 staining was detected in adventitia tissue, probably reflecting the high level of expression of SCD1 in the perivascular adipose tissue.

Many developmental, dietary, hormonal and environmental factors such as high-carbohydrate diets, glucose and fructose, polyunsaturated fatty acids, insulin, and cholesterol have been found to regulate SCD1 expression [18–21]. Thus, the regulation of SCD is very diverse and its expression could affect many key physiological variables and diseases, including insulin sensitivity, metabolic rate, adiposity, atherosclerosis, and cancer [5,8,22,23]. In this study, we have provided novel evidence that SCD1 is up-regulated by laminar shear stress. This, to our knowledge, is the first report demonstrating that the expression of SCD1 is regulated by a mechanical stimulus. Our result has furthered the current understanding of the regulation of SCD1 and its implication in physiological metabolic processes and disease conditions. The flow patterns in the arterial tree are complex. The interplay of the cardiac cycle and the vessel geometry results in the pulsatile nature of blood flow and the asymmetric shape of the velocity profile, thus leading to temporal and spatial variations of wall shear stress. In the straight part of arterial tree, the blood flow is relatively undisturbed. However, the blood flow patterns in bends and bifurcations are disturbed with eddies [24–26]. Our in vivo immunohistochemical results showed that the expression of SCD1 in endothelium is continuous in the straight parts of rat abdominal aorta and the medial aspect of the celiac branch, where the ECs are exposed to a higher undisturbed pulsatile shear stress. However, SCD1 expression was markedly reduced in the endothelium on the lateral aspect of the branch, where secondary flow and flow separation are more likely to occur [24–26]. These results are in agreement with our in vitro findings and demonstrate the ability of laminar flow to increase the expression of SCD1 in ECs. Shear stress is a major regulator of endothelial function and metabolism. Laminar shear stress has been known to increase the membrane fluidity in ECs. The alteration of membrane fluidity may, in turn, impose significant impacts on multiple cell signaling pathways. Since the lipid composition of cellular membranes is regulated to maintain membrane fluidity, the membrane-bound SCD1 is a key enzyme involved in this process. The principal product of SCD1, oleic acid, accounts for increased membrane fluidity. In fact, enriching the cell membrane with cholesterol significantly reduced membrane fluidity in ECs [27]. In contrast, the cell membrane isolated from the SCD1−/− mice is very rigid because of decreased membrane fluidity [24,25,28]. Similarly, an increase in SCD protein content is paralleled by increased membrane fluidity [29]. Therefore, we postulate that the shear-induced expression of SCD1 may be responsible for the maintenance of a proper membrane fluidity of ECs and contribute to the anti-atherosclerotic effects of laminar flow in vessels.

In this study, we found that the flow induction of SCD1 is mediated via a PPARγ specific pathway, which is supported by in vitro and in vivo evidence. In particular, both basal and flow-associated SCD1 expressions in endothelium were markedly reduced after treatment with BADGE, which is used as a selective antagonist of PPARγ [10]. PPARs are a family of ligand-activated transcription factors and consist of three isoforms: α, β/δ and γ [30]. PPARγ is activated by various endogenous and synthetic ligands, including TZDs. Although primarily expressed in adipose tissue, PPARγ is...
also present in the vasculature, including ECs, and has been implicated in a number of vascular pathological processes such as atherosclerosis and vascular remodeling [31,32]. It has been recently reported that laminar flow activates PPARγ in ECs [10]. The molecular mechanisms linking shear stress and PPARγ activation remain to be fully explored. We have previously shown that shear stress activates PPARγ via a ligand-dependent manner, likely, by increasing the production of its endogenous ligand [10]. Subsequently, it has been demonstrated that epoxygenase P450 epoxygenases, are ligands for PPARγ and mediate the flow activation of PPARγ. PPARγ may mediate the anti-inflammatory effect of laminar flow in ECs [16]. Here, our study further suggested that PPARγ might also mediate the pro-metabolic effects of laminar flow, via the induction of SCD1. The role of PPARs in regulation of SCD expression has been previously examined. In 3T3-L1 adipocytes, troglitazone and pioglitazone were found to decrease SCD1 mRNA levels [33,34]. However, in Zucker diabetic fatty rats, SCD1 mRNA was induced in both adipose and skeletal muscle tissues by rosiglitazone [35]. Similarly, overexpression of a constitutively active PPAR-γ2 also resulted a robust induction of SCD1 mRNA in 3T3-L1 adipocytes, indicating that SCD1 is a PPAR-γ target in adipocytes [36]. These divergent results were likely due to differences in the TZDs used or in the tissues and animal models studied. Recently, Reser and colleagues, in a randomized and placebo-controlled study of the patients with type 2 diabetes, found that mRNA expression of SCD was increased after a 3-month treatment with rosiglitazone. In addition, an increase in SCD activity index was associated with improved insulin sensitivity [37]. We found that TZDs up-regulated SCD1 expression at both the mRNA and protein levels in ECs and that the TZD-mediated up-regulation was blocked by the PPARγ antagonist GW9662. Furthermore, overexpression of a constitutively active PPAR-γ was sufficient to induce the SCD1 expression. Taken together, these results support the concept that SCD1 is positively regulated by PPAR-γ activation in ECs. Since a consensus PPAR-responsive element was previously identified in the promotor region of the SCD1 gene to mediate the induction by PPARα agonists clofibrate and gemfibrozil in mouse liver [38], it is plausible that TZDs may directly regulate the transcription of SCD1 by recognizing the PPRE motif. This is also consistent with a previous observation that PPARα-deficient ob/ob mice had a reduction of SCD gene expression [39].

SCD1 plays an important role in many diseases of altered cellular metabolism including obesity, insulin resistance, and dyslipidemia. A recent study revealed a critical role of SCD1 in hepatic insulin resistance [5]. However, little has been known about the role of SCD1 in peripheral tissues such as vascular ECs. In contrast to a generally expected detrimental role in adipose and hepatic tissues, SCD1 may also provide a protective role in peripheral non-lipogenic tissues such as vascular ECs. As discussed earlier, the flow-induced SCD1 may lead to a sustained increase in membrane fluidity and modulate the EC responses to a variety of pro-atherogenic stimuli. In addition, increased SCD1 expression in ECs may exert beneficial effects through at least two other mechanisms. First, SCD1 desaturates stearic acid and converts it into oleic acid, which potently suppresses endothelial activation by inhibiting the expression of pro-inflammatory cytokines and adhesion molecules [40]. Second, in hyperlipidemic states, the accumulation of excess lipids in non-adipose tissues leads to cell dysfunction and apoptosis, known as lipotoxicity. Since lipotoxicity from accumulation of long chain fatty acids is specific for saturated but not unsaturated fatty acids [41,42], increased SCD expression may provide an enhanced endothelial defense against lipotoxicity in response to lipid overload in hyperlipidemia by modulating the intracellular ratio of unsaturated to saturated fatty acids. Thus, the flow induction of SCD1 in ECs may represent a metabolic response and play an athero-protective role. However, our present study has only revealed the gene regulation of SCD1 and a transcriptional mechanism by laminar flow. Further studies examining the specific activity of SCD1 and its contribution to alterations in lipid composition, membrane fluidity and EC functions will be helpful in elucidating the functional roles of the flow-mediated regulation of SCD1.

In conclusion, our results show that laminar flow induced the expression of SCD1 gene and protein in vascular ECs, which is mediated by the flow-activation of the PPAR-γ pathway. Since SCD1 is a critical enzyme in controlling cell metabolism and lipid composition, these results may provide new insights into the mechanisms underlying the multiple athero-protective effects of laminar flow, including increased endothelial membrane fluidity and improved endothelial function.

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