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RESEARCH PAPER

Distinct regulation of stearoyl-CoA desaturase 1 gene expression by *cis* and *trans* C18:1 fatty acids in human aortic smooth muscle cells

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Abstract Consumption of *trans* fatty acids is positively correlated with cardiovascular diseases and with atherogenic risk factors. *Trans* fatty acids might play their atherogenic effects through lipid metabolism alteration of vascular cells. Accumulation of lipids in vascular smooth muscle cells is a feature of atherosclerosis and a consequence of lipid metabolism alteration. Stearoyl-CoA desaturase 1 (scd1) catalyses the production of monounsaturated fatty acids (e.g. oleic acid) and its expression is associated with lipogenesis induction and with atherosclerosis development. We were interested in analysing the regulation of delta-9 desaturation rate and scd1 expression in human aortic smooth muscle cells (HASMC) exposed to *cis* and *trans* C18:1 fatty acid isomers (*cis*-9 oleic acid, *trans*-11 vaccenic acid or *trans*-9 elaidic acid) for 48 h at 100 μM. Treatment of HASMC with these C18:1 fatty acid isomers led to differential effects on delta-9 desaturation; oleic acid repressed the desaturation rate more potently than *trans*-11 vaccenic acid, whereas *trans*-9 elaidic acid increased the delta-9 desaturation rate. We then correlated the delta-9 desaturation rate with the expression of scd1 protein and mRNA. We showed that C18:1 fatty acids controlled the expression of scd1 at the transcriptional level in HASMC, leading to an increase in scd1 mRNA content by *trans*-9 elaidic acid treatment, whereas a decrease in scd1 mRNA content was observed with *cis*-9 oleic acid and *trans*-11 vaccenic acid treatments. Altogether, this work highlights a differential capability of C18:1 fatty acid isomers to control scd1 gene expression, which presumes of different consequent effects on cell functions.

Keywords Stearoyl-CoA desaturase · *trans* fatty acids · Oleic acid · Vascular smooth muscle cells · Atherosclerosis

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Introduction

Consumption of *trans* fatty acids has been associated with higher risk of cardiovascular diseases (Chiuve et al. 2009; Oh et al. 2005; Oomen et al. 2001). The main *trans* fatty acids in foods are *trans*-9 elaidic acid (C18:1 *trans*-9) and *trans*-11 vaccenic acid (C18:1 *trans*-11) found in hydrogenated vegetable oils and ruminant-derived products, respectively (Bauman et al. 2006). Although *trans* fatty acid intakes have been related to pro-atherogenic risks, no cause-consequence effects were firmly evidenced between their consumption and atherosclerosis (Mozaffarian et al. 2006). However, high content of *trans*-9 elaidic acid (ELA) has been observed in atheroma plaques and adipose tissue of obese subjects (Bortolotto et al. 2005; Stachowska et al. 2004). A recent report demonstrated that *trans* fatty acid from hydrogenated vegetable oil-enriched diet (mainly *trans*-9 elaidic acid) enhanced the development of atheroma lesions in $LDLr^{-/-}$ mice (Bassett et al. 2009). In contrast, *trans*-11 vaccenic acid (VA) would be able to protect against atherosclerosis development (Bassett et al. 2010).

Endothelial and vascular smooth muscle cells (VSMC) are the major cell types of the vascular wall, and the development of atherosclerosis is dependent on the modification of their phenotype (e.g. inflammatory status, apoptosis, proliferation). Thus, elaidic acid would stimulate atherogenesis through endothelial dysfunction and

alteration of lipid metabolism (Mozaffarian et al. 2006; de Roos et al. 2001; Zapolska-Downar et al. 2005). VSMC also play a preponderant role in the pathogenesis of atherosclerosis by their migration and proliferation in the intima of arterial wall, where they accumulate lipids and contribute to the promotion of inflammation process (Orr et al. 2010). Accumulation of lipid in VSMC is associated to either uptake of lipoproteins and fatty acids from plasma, or de novo lipogenesis with increased expression of lipogenic genes such as stearoyl-CoA desaturase (scd) (Leake and Peters 1982; Mietus-Snyder et al. 1997; Portman 1970; Ricciarelli et al. 2000; Davies et al. 2005; Hamlat et al. 2009). The stearoyl-CoA desaturase localizes in the endoplasmic reticulum and exists under two isoforms scd1 and scd5 in human (Wang et al. 2005). Scd plays its central role in partitioning endogenous and dietary fatty acids into metabolically active or inactive pools and is a key enzyme of mono-unsaturated fatty acid (MUFA) biosynthesis. Scd1 requires the presence of NADH-reductase, cytochrome b5 and oxygen to catalyse the introduction of a *cis* double bound at delta-9 position of palmitoyl- or stearoyl-CoA to form palmitoleyl- or oleyl-CoA, respectively (Shimakata et al. 1972). Thus, most oleic acid is synthesized from endogenous delta-9 desaturation of C18:0. Moreover, scd1 also metabolized *trans* fatty acids: vaccenic acid into *cis*-9, *trans*-11 conjugated linoleic acid and elaidic acid has been shown to interfere with oleic acid synthesis (Pollard et al. 1980; Lock et al. 2004).

The understanding of how *trans* fatty acids modulate scd1 expression and activity in VSMC would help to better define the possible contribution of these *trans* fatty acids to atherosclerosis. In this purpose, we compared the influence of *cis* and *trans* C18:1 fatty acid isomers on regulation of expression of scd1 in human aortic smooth muscle cell (HASMC).

Materials and methods

Materials

Human aortic smooth muscle cells (HASMC) were obtained from Invitrogen (Cergy-Pontoise, France). DMEM without phenol red, delipidated foetal bovine serum, penicillin, streptomycin and L-glutamine were purchased from Dutscher (France) and medium 231 supplemented with smooth muscle growth supplement (SMGS) from Invitrogen. Anti-scd1 was purchased from Santa-Cruz biotechnologies and anti-β-actin from Sigma. Oleic, stearic, *trans*-11 vaccenic and *trans*-9 elaidic acids were obtained from Sigma and [^{14}C] stearic acid from Perkin-Elmer (Courtaboeuf, France). CyQuant® NF cell proliferation assay was obtained from Invitrogen.

Cell culture conditions

HASMC were maintained in medium 231 supplemented with SMGS (Invitrogen), penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). For experiments, cells were maintained for 24 h in DMEM without phenol red, supplemented with 1% of delipidated FBS, 0.5% of fatty acid free-bovine serum albumin (BSA) (Sigma), penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). Subsequently, cells were treated with *cis*-9 oleic, *trans*-11 vaccenic or *trans*-9 elaidic acids bound to BSA (ratio 4:1) at a final concentration of 100 µM for 48 h.

Cell proliferation assay

Cell proliferation analysis was carried out using CyQuant® NF cell proliferation assay (Invitrogen). Adherent cells were washed and frozen at −80°C. Cells were then thawed and treated with CyQuant GR dye, which binds stochiometrically to nucleic acids. Fluorescence quantification was measured using a microtiter plate fluorimeter (VICTOR^{3}V™, PerkinElmer Life Sciences Inc., Wellesley, MA) with excitation at 485 nm and detection at 535 nm.

Fatty acid analysis and scd index

Cells were treated with fatty acids at 100 µM for 48 h as previously described. Lipid extraction was performed by a Folch modified method (Bellenger-Germain et al. 2002) and followed by saponification/methylation. Methyl esters were analysed by gas chromatography (GC) on Clarus 500 (Perkin-Elmer) chromatograph equipped with on-column injector and FID detector. The GC was performed on capillary column VF23 ms 60 M × 0.32 MM (Varian) using hydrogen gas; oven temperature was programmed from 60 to 148°C at 35°C/min and then to 210°C at 1.5°C/min.

The scd desaturation index was assessed as 16:1 n-7/16:0 and 18:1 n-9/18:0.

Determination of delta-9 desaturation rate

Conversion of [^{14}C] stearic acid into [^{14}C] oleic acid was used to measure scd activity after 48 h in presence of C18: 1 fatty acids. Cells were incubated with 3 µM of [^{14}C] stearic acid (0.25 µCi/dish) for 6 h at 37°C in 5% CO_2 incubator. Cells were then collected, and total lipids were extracted according to Bligh and Dyer method for saponification and esterification. Radiolabelled fatty acid methyl esters were then separated by RP-HPLC and detected on line by a radioisotope detector (Packard Flow Scintillation Analyser, PerkinElmer Life Sciences Inc., Wellesley, MA) (Narce et al. 1992). The [^{14}C] oleic acid/([^{14}C] oleic and stearic acids) ratio was defined as desaturation rate by scd activity.

Western blotting

Cells were washed with ice-cold PBS and lysed in ice-cold Ripa buffer containing protease inhibitor cocktail (Sigma) for 15 min on ice. We cleared protein lysate by centrifugation at 10,000×*g* for 10 min at 4°C. Thirty micrograms of total proteins were loaded for SDS-PAGE electrophoresis and transferred onto a nitrocellulose membrane. Immunoblotting was performed with antibodies raised against scd1 and *β*-actin. Immunoreactive bands were detected using horseradish peroxidase-conjugated secondary antibody.

RNA purification and RT-qPCR

Total RNA was purified using RNEasy kit (Qiagen, Germany). Reverse transcription was performed with 1 µg RNA using iScript cDNA Synthesis Kit (Bio-Rad).

RNA expression level was quantified by real-time PCR using iQ SYBR Green supermix (Bio-Rad) using a Bio-Rad iCycler iQ with the following primers:

Scd1: sense 5′-TTC AGA AAC ACA TGC TGA TCC TCA TAA TTC CC-3′ and antisense 5′-ATT AAG CAC CAC AGC ATA TCG CAA GAA AGT CTG-3′; and *β*-actin: sense 5′-CTG GTG CCT GGG GCG-3′ and antisense 5′-AGC CTC GCC TTT GCC GA-3′. The relative amount of target genes was determined using $\Delta\Delta C$t method. The normalized delta cycle threshold (ΔCt) was calculated by subtracting the gene cycle threshold value from the *β*-actin cycle threshold value ($\Delta C\text{t} = C\text{t}_{\beta\text{-actin}} - C\text{t}_{\text{gene}}$). Comparative gene expression between two independent samples, or $\Delta\Delta C$t, was obtained by subtracting the control delta cycle threshold from the sample delta cycle threshold ($\Delta\Delta C\text{t} = \Delta C\text{t}_{\text{sample}} - \Delta C\text{t}_{\text{control}}$). Fold change expression was defined with $2^{-(\Delta\Delta C\text{t})}$.

Luciferase reporter assay

HASMC were transiently co-transfected with −500 bp human scd1 promoter-PGL3 (Bene et al. 2001) and *β*-galactosidase expression vector (Clontech) for normalization using Amaxa® human AoSMC Nucleofector® (Lonza) according to the manufacturer recommendations. Twenty-four hours after transfection, cells were treated with fatty acids as described above. Then, cells were collected and lysed with Reporter Lysis Buffer (Promega) for luciferase and *β*-galactosidase activity analysis.

Statistical analysis

Results are presented as means ± standard error mean (SEM). Statistical significance of results was determined by Oneway Anova analysis followed by Tuckey's HSD test. $P < 0.05$ was considered significant.

Results

C18:1 fatty acid isomers do not modify HASMC growth

We first defined the concentration of fatty acids and the time of exposure from the oleic acid (OA) effect on scd1 protein expression. Thus, we showed that human aortic smooth muscle cells (HASMC) exposed to 50 and 100 µM OA for 48 h presented a decrease in scd1 expression in a dose–response manner (Fig. 1a). We also found that HASMC treatment with OA at 100 µM down-regulated scd1 expression in a time-dependent manner with a very pronounced effect at 48 h (Fig. 1b). On this basis, we chose these experimental conditions for all fatty acids.

We then compared the effect of OA to *trans* C18:1 fatty acids on cell growth. HASMC were maintained in

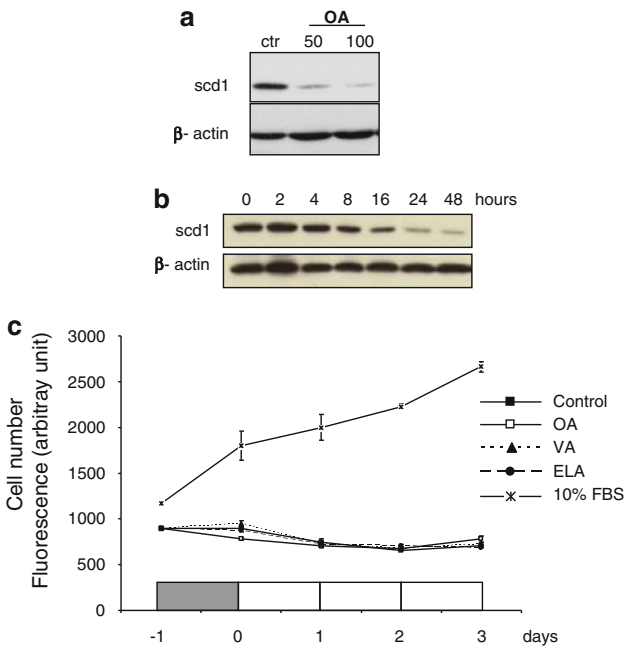

Fig. 1 HASMC growth in the presence of C18:1 fatty acid isomers. **a** HASMC were pre-treated for 24 h in DMEM with 0.5% BSA and then exposed to control medium (ctr), 50 and 100 µM of oleic acid (OA) for 48 h. Protein lysates were prepared for the analysis of scd1 protein expression by Western blotting. **b** HASMC were pre-treated for 24 h in DMEM with 0.5% BSA and then treated with 100 µM of oleic acid. Cells were collected at various time points for analysis of scd1 protein expression by Western blotting. **c** HASMC were pre-treated for 24 h in DMEM with 0.5% BSA. At day 0, cells were treated with oleic acid (OA), *trans* vaccenic acid (VA) and elaidic acid (ELA) at 100 µM. Proliferation control cells were maintained in DMEM with 10% FBS. Cell number was determined by Cyquant® kit. Values represent the mean ± SEM ($n = 3$)

quiescent state in serum-free medium for 24 h before fatty acid treatment. We incubated HASMC with 100 μM oleic acid, *trans*-11 vaccenic acid (VA) or *trans*-9 elaidic acid (ELA) bound to BSA or BSA alone (0.5%) as control for indicated time. We observed that the treatment with these different FA did not modify the HASMC growth thereby excluding misinterpretation of scd activity regulation due to different cellular growth state (Fig. 1c).

C18:1 fatty acid isomers differentially modulate scd index

We determined the C16:1n-7/C16:0 and C18:1n-9/C18:0 ratio referred as scd index which might reflect the scd activity. We found that VA and ELA treatment increased C16:1n-7/C16:0 ratio compared to OA and control treatments (Fig. 2a). In OA-treated HASMC, the addition of exogenous OA (C18:1 n-9) led to a dramatically increase in C18:1n-9/C18:0 ratio compared to the control, VA- and ELA-treated HASMC. Moreover, we observed that ELA treatment more modestly than OA treatment increased C18:1n-9/C18:0 ratio compared to the control treatment (Fig. 2b). Altogether these results suggested that the delta-9 desaturation capability of scd could be differently regulated by the C18:1 fatty acid isomers. In order to evaluate the delta-9 desaturation rate in HASMC treated with the different C18:1 fatty isomers, we analysed [^{14}C] stearic acid conversion into [^{14}C] oleic acid in cells exposed to 100 μM of C18:1 fatty acid (OA, VA or ELA) for 48 h. Compared to control cells, the delta-9 desaturation rate was decreased in a modest manner in VA-treated HASMC, while a dramatic decrease was observed in OA-treated HASMC (Fig. 2b). By contrast, ELA treatment increased the C18:0 desaturation rate up to 80% (Fig. 2b).

C18:1 fatty acid isomers differentially regulate scd1 protein expression in HASMC

The changes in delta-9 desaturation rate induced by C18:1 fatty acid isomers led us to analyse scd1 protein expression in HASMC treated with *cis* and *trans* C18:1 FA isomers. We found that scd1 protein content in HASMC was dramatically decreased with OA treatment for 48 h compared to the control cells (Fig. 3). We also observed that HASMC exposed to ELA presented an increase in scd1 protein expression, whereas a slight decrease in scd1 expression was shown in VA-treated HASMC (Fig. 3).

The expression of scd1 protein appeared to be in agreement with the capability of HASMC to desaturate the stearic acid in presence of the different C18:1 isomers.

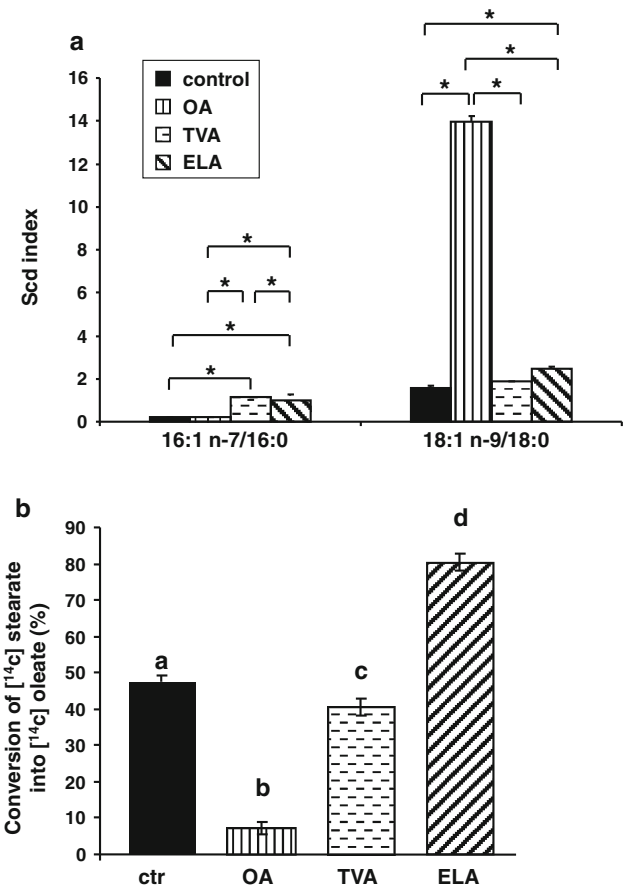

Fig. 2 Scd index and delta-9 desaturation of HASMC treated with C18:1 fatty acid isomers. **a** HASMC were treated with 100 μM of oleic acid (OA), *trans*-11 vaccenic acid (VA) or *trans*-9 elaidic acid (ELA) for 48 h and total fatty acid composition was analysed by gas chromatography. Scd index was determined as C16:1n-7/C16:0 or C18:1 n-9/C18:0. Values are the mean ± SEM of three independent experiments. *Significantly different ($P < 0.05$). **b** Determination of delta-9 desaturation rate in HASMC exposed to 100 μM of fatty acid compared to control (ctrl) HASMC. HASMC treated with C18:1 isomers for 48 h were incubated with [^{14}C] stearic acid for further 6 h, and total lipid extraction was performed for radiolabelled fatty acid separation by RP-HPLC. *Values* represent the mean ± SEM of three independent experiments. *Bars* assigned different superscript letters (*a*, *b*, *c* and *d*) were significantly different ($P < 0.05$)

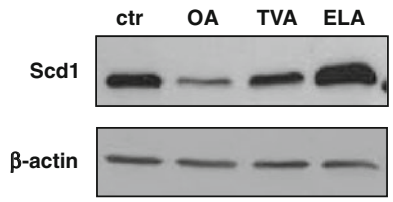

Fig. 3 Expression of scd1 protein is regulated by C18:1 fatty acid isomers. HASMC were treated for 48 h at 100 μM with oleic acid (OA), *trans*-11 vaccenic acid (VA), *trans*-9 elaidic acid (ELA) or BSA (ctr). HASMC were prepared for whole protein extract and scd1 expression was analysed by Western blotting

C18:1 fatty acid isomers control scd1 expression at transcriptional level in HASMC

We were then interested in analysing the regulation of scd1 mRNA expression by RT-qPCR in HASMC treated for 48 h with 100 μM of C18:1 FA isomers. We found that OA treatment drastically reduced scd1 mRNA expression compared to control and *trans* C18:1 FA treatments. Concerning *trans* FA, VA slightly decreased scd1 mRNA expression, whereas ELA treatment increased it (Fig. 4a). To determine whether the regulation of scd1 mRNA content was at transcriptional level, we analysed the activity of human scd1 promoter-luciferase vector transfected in HASMC treated with C18:1 FA. We demonstrated that the treatments with the different fatty acids changed significantly ($P < 0.05$) the scd1 promoter activity compared to the control HASMC: OA and VA repressed scd1 promoter activity about approximately 40 and 20 %, respectively, in comparison with control treatment, whereas ELA led to an increase in scd1 promoter activity about 20% (Fig. 4b). Furthermore, the scd1 promoter activities observed in HASMC treated either by OA, TVA or by ELA were different and reached statistical significances ($P < 0.05$). These observations showed that C18:1 FA regulated transcriptional activity of scd1 gene in HASMC.

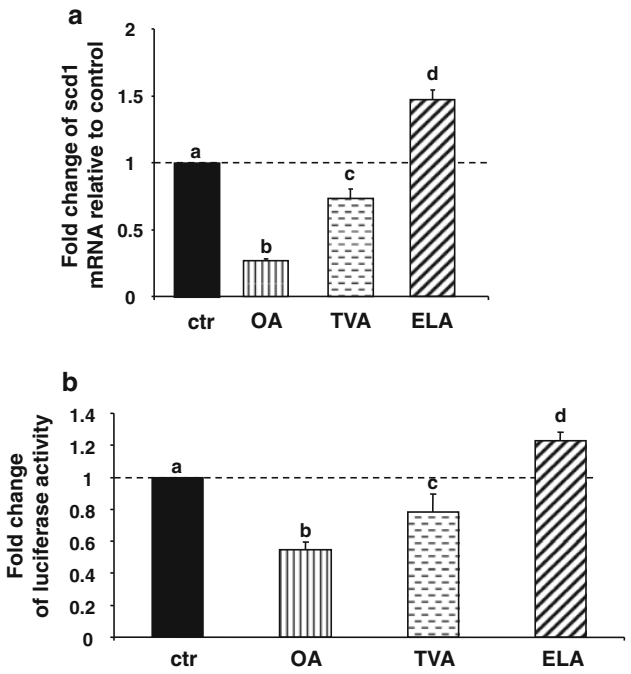

Fig. 4 Regulation of scd1 transcription by C18:1 fatty acids in HASMC. **a** Analysis of scd1 mRNA expression by RT-qPCR in HASMC treated for 48 h at 100 μM oleic acid (OA), *trans* vaccenic acid (VA) and *trans* elaidic acid (ELA) compared to control BSA (ctr). **b** Quantification of human scd1 promoter luciferase activity normalized with *β*-galactosidase expression vector activity in HASMC treated with OA, VA and ELA at 100 μM for 48 h compared to control cells (ctr). Values represent the mean ± SEM of three independent experiments. *Bars* assigned different superscript letters (*a*, *b*, *c* and *d*) were significantly different ($P < 0.05$)

Discussion

During atherosclerosis, proliferation and apoptosis of aortic smooth muscle cells play a preponderant role in the progression and the vulnerability of atheroma plaque (Clarke et al. 2006; Dzau et al. 2002). Some fatty acids are able to modulate both cellular parameters according to their carbon chain length and saturation degree (Artwohl et al. 2009; Lu et al. 1996; Shiina et al. 1993). In the present study, we observed that *cis* and *trans* C18:1 fatty acid isomers did not promote neither proliferation nor apoptosis of quiescent HASMC (Fig. 1c). In contrast, oleic acid is able to enhance proliferation of HASMC induced by promitogenic factors (data not shown and Renard et al. 2003). Scd1 expression is associated with the regulation of survival and proliferation signals in normal and cancer cells. Decrease in scd1 expression triggered either apoptosis in cancer cells or increase in palmitate-induced cytotoxicity in endothelial and pancreatic beta cells (Green and Olson 2011; Minville-Walz et al. 2010; Peter et al. 2008). We demonstrated here that the down-regulation of stearoyl-CoA desaturase expression and activity with oleic acid (OA) or *trans*-11 vaccenic acid (VA) treatment did not affect viability of HASMC. These observations suggested that HASMC present a different behaviour than cancer cells when scd activity is reduced, all the more so HASMC treated with VA produced *cis*-9 *trans*-11 conjugated linoleic acid (data not shown) known as a cytotoxic fatty acid for colon and breast cancer cells (Miller et al. 2003).

The down-regulation of scd1 mRNA expression by OA has also been described in rat hepatocytes (Landschulz et al. 1994). However, this regulation of scd1 mRNA has not been observed in mouse adipocytes (3T3-L1) or human hepatocarcinoma cells (HepG2) highlighting a scd1 regulation way by OA depending on the cell type (Bene et al. 2001; Sessler et al. 1996). Moreover, scd1 mRNA expression is decreased more drastically when hepatocytes are treated with fatty acids of higher unsaturation degree (Landschulz et al. 1994). Landschulz et al. (1994) showed that linolenic acid (18:3) was a more potent suppressor of scd1 mRNA expression than linoleic (18:2) and oleic (18:1) acids and that FA with identical unsaturation number exerts similar repression of scd1 mRNA expression. In this study, we were not able to conclude on a link between the regulation of scd1 expression and the *cis*/*trans* configuration or the position of the unsaturation but we evidenced that fatty acids with same carbon chain length (C18) and same unsaturation degree could differently modulate scd1

expression. Moreover, we can postulate that the repression of scd1 gene transcription by OA and more modestly by VA treatment involved a negative feed-back loop by end-product of scd activity in HASMC. Indeed, the stearoyl-CoA desaturase catalyses the transformation of stearic acid into oleic acid and *trans*-11 vaccenic acid into *cis*-9 *trans*-11 conjugated linoleic acid (Pollard et al. 1980). It would have been interesting to define the scd1 regulation by substrates of scd1 (e.g. palmitic and stearic acids) in order to analyse the effect of endogenous synthesis of their respective products—palmitoleic and oleic acids—on scd1 expression. Furthermore, effect of exogenous *cis*-11 vaccenic acid on scd1 function would also have been of interest since this fatty acid is a positional isomer of oleic acid without being produced by scd1.

Scd1 expression may be regulated at different cellular levels from transcriptional to post-translational level (Ntambi and Miyazaki 2004). In this study, we evidenced that *cis* and *trans* C18:1 fatty acids controlled the delta-9 desaturation rate in HASMC by the regulation of scd1 gene transcription. Indeed, we showed that human scd1 promoter activity was modulated by FA treatment and correlated with scd1 mRNA expression indicating that scd1 mRNA content was primarily regulated by C18:1 isomers at transcriptional level in HASMC. The human scd1 promoter construct used for this study contains regulatory elements for transcription factors such as the sterol regulatory element-binding protein (SREBP), CCAAT enhancer binding protein alpha (C/EBPα) and nuclear factor-1 (NF-1) (Bene et al. 2001). Different transcription factors participate to the regulation of scd1 gene expression by dietary factors (e.g. PUFAs, cholesterol) or hormones (e.g. insulin, leptin) (Mauvoisin and Mounier 2011). However, SREBP-1 transcription factor appears as the main regulator in this context. Thus, we postulated that modulation of scd1 transcription involved SREBP-1. We were not able to show any modifications of SREBP-1 expression or maturation (data not shown) that would exclude a regulation of scd1 gene transcription by oleic, vaccenic and elaidic acids through this pathway. SREBP-1 independent regulation has been described and might involve LXR pathway. Indeed, direct binding of LXR to mouse scd1 promoter occurred and a treatment with an LXR agonist increased scd1 transcription in SREBP-1C knock-out mice (Chu et al. 2006; Liang et al. 2002). Thus, a regulation of scd1 gene expression by MUFA might be mediated through the modulation of LXR signalling. Moreover, the NF-Y transcription factor has also been reported for its essential role in either the activation by sterol depletion or the inhibition by PUFA of scd1 promoter activity (Tabor et al. 1999; Teran-Garcia et al. 2007). The NF-Y response element is found in scd1 promoter, and the C18:1 fatty acids might play their effect by a regulation of NF-Y binding to the promoter (Tabor et al. 1999). The control of scd1 gene expression by upstream signalling would involve modulation of Erk1/2 activation. Indeed, increase in Erk1/2 phosphorylation by leptin has been associated to a decrease in scd1 transcription in HepG2 (Mauvoisin et al. 2010). Thus, down-regulation of scd1 gene expression by OA might also be induced by its capability to activate Erk1/2 already described in VSMC (Zhang et al. 2007).

In summary, we have observed that C18:1 fatty acids differentially regulated scd activity and scd1 expression in HASMC. Although we demonstrated that scd1 expression was regulated by oleic acid in a time-dependent and in a dose–response manner, we did not evaluate these two parameters for the *trans* fatty acids that might be somewhere limitative. However, we clearly showed at 100 μM for 48 h that oleic and *trans*-11 vaccenic acids repressed both scd activity and scd1 expression, while *trans*-9 elaidic acid enhanced scd1 activity and expression.

Such, modulation of scd activity under the influence of dietary C18:1 fatty acids might account for changes in vascular smooth muscle cell fate during atherogenesis and further investigations need to be undertaken to precise the role of scd1 expression regulation in these cells.

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