

Lipid Rafts Promote *trans* Fatty Acid-Induced Inflammation in Human Umbilical Vein Endothelial Cells

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Abstract The effects of two fatty acids, oleic acid (OLA) and elaidic acid (ELA) on normal human umbilical vein endothelial cells (HUVEC) and non-rafts HUVEC were investigated in this study. The expression levels of inflammatory cytokines (ICAM-1, VCAM-1 and IL-6) were analyzed. Western blot was used to analyze the expression levels of inflammation-related proteins (NF- κ B, ERK1/2) and toll-like receptors 4 (TLR4). The results showed that the levels of nuclear translocation of NF- κ B p65 and phosphorylated ERK1/2 were significantly decreased only in non-lipid rafts cells pretreated with *trans* fatty acid (TFA). The expression of TLR4 in the ELA-treated normal cells was higher than that in non-lipid rafts HUVEC. When the lipid rafts was destroyed by methyl- β -cyclodextrin, the levels of nuclear translocation of NF- κ B p65, phosphorylated ERK1/2 and TLR4 were decreased significantly. Therefore, lipid rafts may be involved in TFA induced-inflammation in HUVEC through blocking the inflammatory signal pathway. Lipid rafts might be a platform for specific receptors such as TLR4 for TFA to activate the pro-inflammation on cell membranes.

Keywords *trans* Fatty acids · Lipid rafts · Inflammation · NF- κ B · ERK1/2

Abbreviations

DMEM Dulbecco's modified eagle medium

ELA	Elaidic acid
EC	Endothelial cell(s)
FBS	Fetal bovine serum
HUVEC	Human umbilical vein endothelial cell(s)
ICAM-1	Intercellular adhesion molecule-1
IL-6	Interleukin-6
M β CD	Methyl- β -cyclodextrin
OLA	Oleic acid
PBS	Phosphate buffered saline
TFA	<i>trans</i> Fatty acid(s)
TLR4	Toll-like receptor 4
VCAM-1	Vascular cell adhesion molecule-1

Introduction

In the US population, the consumption of *trans* fatty acids (TFA) consist of more than 4% of the daily dietary fat intake [1, 2]. The over intake of TFA that contain at least one carbon-carbon *trans* double bond might lead to atherosclerosis, insulin resistance, obesity, hypertension and type 2 diabetes [3]. TFA was reported to be linked to systemic inflammation, endothelial dysfunction, insulin resistance and adiposity resistance [4]. Both randomized control trials and observational studies found that TFA promoted vascular diseases mainly through accelerating inflammation and endothelial dysfunction [3, 5, 6]. For example, TFA was reported to be associated with the plasma biomarkers of inflammation, increased expression levels of soluble intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1) and E-selectin [7]. Moreover, NF- κ B pathway, including p65 (RelA), could control various biological events, such as immune responses, cell growth and survival [8, 9]. The activation of ERK pathway was

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implicated in a wide variety of cellular functions as diverse as cell proliferation, cell-cycle arrest, terminal differentiation and apoptosis [10]. Endothelial cells (EC) are critical for the development and progression of atherosclerosis. TFA was also reported to induce EC apoptosis through caspase pathway [11] and promote the release of endothelial inflammatory cytokines [3]. Caspase-3-mediated p65 cleavage is believed to suppress NF- κ B-mediated anti-apoptotic transactivation in cells undergoing apoptosis [12]. In addition, previous studies showed that NF- κ B was implicated in inducible expressions of a variety of genes, including those encoding for adhesion molecules, such as ICAM-1 and VCAM-1 [13]. It was suggested that a lot of anti-inflammatory effects were through the modification of lipid rafts and secondary effects on NF- κ B signaling [14]. The most abundant form of the protein is a heterodimer of p50 and p65 subunits, in which the p65 subunit contains the transcriptional activation domain [8]. It was also reported that the association of NF- κ B with lipid rafts was dependent upon phosphorylation of NF- κ B p65 and lipid raft plays an essential role in NF- κ B transcriptional activity [14]. A previous study showed that *trans*-10 conjugated linolenic acid mediated dedifferentiation and delipidation of newly differentiated human adipocytes were associated with MEK/ERK signaling through the autocrine/paracrine actions of interleukin-6 and -8 [15]. Others [16] reported that 9*c*11*t* conjugated linoleic acid (CLA) changed cell membrane structure and then interfered with intracellular transport of epidermal growth factor receptor and its related signaling pathway including ERK, both functionally associated with lipid raft properties. Hence, we hypothesize that TFA may lead the EC inflammation by activating the NF- κ B signal pathway and ERK1/2 pathway. However, it is still unknown how the signal pathway is activated and whether there exist specific receptors at cell membranes. Lipid rafts, specialized membrane microdomains and enriched with cholesterol and sphingolipids, were reported to play an important role in cell signal transduction, such as inflammation, pro-apoptotic and anti-apoptotic effects [17]. There are many membrane receptors located in lipid rafts which could promote apoptosis after activation [18]. TLR4 is responsible for activating the innate immune system [19]. It has also been reported that lipid rafts appeared to provide a platform for the interaction of endosomal TLR with their ligands in macrophages [19, 20]. The saturated fatty acids acylated on lipid A of lipopolysaccharide (LPS) play critical roles in ligand recognition and receptor activation for TLR4, and saturated and polyunsaturated fatty acids reciprocally modulated the activation of TLR4 [21]. It has been reported that loss of TLR4 protected against saturated fat-induced inflammation and insulin resistance and against *trans* fat (TF) induced obesity, inflammation,

and insulin resistance [22]. Others [23] showed that the partially hydrogenated vegetable oil (*trans* diet) intake by the mothers during pregnancy and lactation led to hypothalamic inflammation and an increase in hypothalamic TLR4 expression. Hence, we suspect that TFA could modulate the activation of TLR4 [22, 23]. On the other hand, several dietary fatty acids such as oleic acid, stearic acid, elaidic acid were reported to be major cell membrane components, and involved in immune and inflammatory processes [24]. Elaidic acid (ELA) was reported to cause EC apoptosis, which might be in relation to lipid rafts [25]. ELA (9*t*18:1 1), one of the most abundant dietary TFA [25], is the major *trans* fatty acid found in hydrogenated vegetable oils and occurs in small amounts in caprine and bovine milk (very roughly 0.1% of the fatty acids) and some meats [25]. Oleic acid (OLA), a fatty acid that occurs naturally in various animal and vegetable fats and oils, was classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 9*c*18:1 [26]. In this study, ELA was used as a representative of TFA and the OLA was a negative control.

However, how TFA activates the vascular cell inflammation, and whether TLR4 in lipid rafts is a specific receptor for TFA to activate the pro-inflammation on the cell membranes are unclear. Therefore, TFA was proposed to have some connection with lipid rafts and TLR4 might be a receptor for human umbilical vein endothelial cells (HUVEC) inflammation. The objectives of this study were: (a) to determine whether TFA induce inflammation in HUVEC; (b) to investigate whether lipid rafts affect inflammatory reaction induced by TFA in HUVEC; (c) to explore the influence of inflammatory cell membrane receptors affected by lipid rafts.

Materials and Methods

Materials and Reagents

Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (USA). Rabbit anti-P65, rabbit anti-p42/44, rabbit anti-phospho-p42/44 antibodies were obtained from Cell Signaling (Boston, MA). Methyl- β -cyclodextrin (M β CD) was obtained from Aladdin (Shanghai, China). ELA and OLA were purchased from Sigma (98.5% purity). Phosphate buffered saline (PBS) was provided by Zhongshanjinqiao (Beijing, China).

Cell Culture and TFA Treatment

HUVEC (Medical College of Nanchang University) 1 were maintained in DMEM supplemented with 10% FBS

and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO₂ and 90% air [26]. In order to determine the role of lipid raft on TFA interfering with HUVEC physiology, cells were divided into six groups: Control group (treated with 0.1 M PBS), ELA group (treated with 100 μmol/L 9*t*18:1 for 24 h), OLA group (treated with 100 Nmol/L 9*c*18:1 for 24 h), MβCD group (treated with 10 mmol/L MβCD for 60 min) [27], MβCD + ELA group (incubated with 100 μmol/L 9*t*18:1 for 24 h after cell treated with 10 mmol/L MβCD for 60 min), MβCD + OLA group (incubated with 100 μmol/L 9*c*18:1 for 24 h after cell treated with 10 mmol/L MβCD for 60 min).

Analysis of Gene Expression by Quantitative PCR

Total RNA (2 μg) was extracted with TRIAZOL reagent and subjected to the reverse transcriptase reaction with random decamers as the primers. The levels of mRNAs and the PCR-product were assessed by 7900HT Real-Time PCR System (Applied Biosystems, California) with melting point analysis. Amplification in the 7900HT was for 45 cycles with initial incubation of 10 min at 95 °C for activation of the Taq polymerase. Cycling parameters were 10 s at 95 °C, 10 s at 63 °C and 15 s at 72 °C. Real-Time PCR samples were mixed with SYBR Green Master Mix (Applied Biosystems, Warrington, UK) and gene-specific primers [28]. The following primers were used to in the PCR.

Western Blot Analysis for Inflammation-Related Proteins

HUVEC were incubated with OLA and ELA (100 μg/mL) for 48 h in 6-well culture plates at the density of 5×10^5 cells/well, respectively. Immediately following the incubation, the cells were washed five times with ice-cold PBS and the total protein was extracted by 50 μL cell lysis buffer (1 mL RIPA + 10 NL PMSF). After incubation for 1 h at −4 °C, the sample was centrifuged at 12,000 r/min for 15 min and the supernatant was separated and stored at −80 °C. Proteins (about 80 μg) were separated by 10% SDS-PAGE, and then the separated proteins were transferred to 1 Immobilon-P transfer membranes. Membranes were blocked with 5% inactivated FBS for 6 h at room temperature (25 °C) and then incubated with the primary antibodies overnight at 4 °C. After washed 3 times (5 min each) with Tris buffer and Tween-20 (TBST), the membranes were incubated with horseradish peroxidase-labeled secondary antibodies for 1 h at room temperature (25 °C). Finally, protein bands were visualized by enhanced chemi-luminescence (ECL) detection system [28].

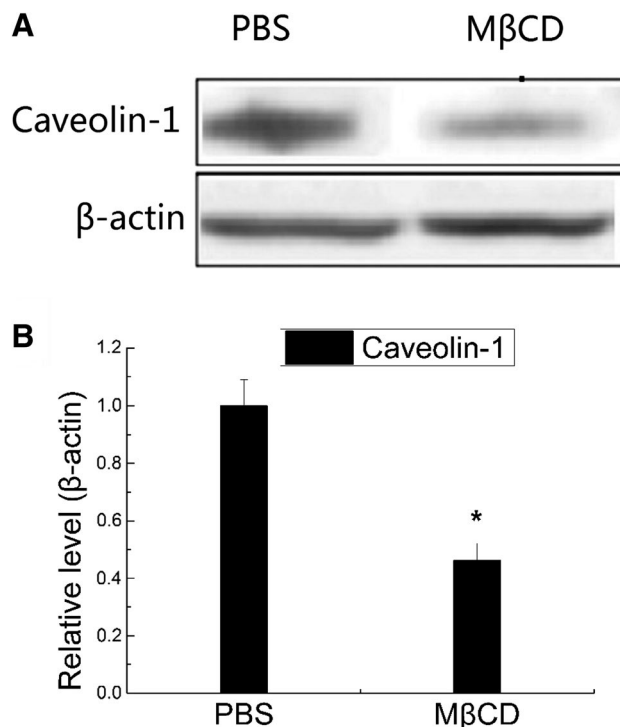


Fig. 1 Western blot analysis of caveolin-1 before and after the pre-treatment of 10 mmol/L MβCD for 60 min (a). The protein levels were quantified by densitometry, and normalized to β-actin levels (b). Data are expressed as means ± SD from three independent experiments. Statistical analysis was carried out using ANOVA. * $p < 0.05$, when compared to the PBS-treated control group

Statistical Analysis

Results were expressed as means ± SD. Data were analyzed using one way analysis of variance (ANOVA), followed by Tukey's *post hoc* comparisons. All statistical analyses were performed with the statistical program SPSS 17.0. * $p < 0.05$, ** $p < 0.01$ was indicative of significant difference.

Results

Effects of MβCD on Lipid Rafts in HUVEC

According to our previous study, HUVEC were treated with MβCD (10 mmol/L) for 60 min to interrupt the function of lipid rafts [26]. In this study, the expression of caveolin-1 in the cells was decreased after treated with MβCD (decreased from 1.00 ± 0.09 to 0.46 ± 0.06 , $p < 0.05$, Fig. 1). This result showed that the structure of lipid rafts was interrupted by MβCD, and the damage of lipid rafts could result from decreased expression of caveolin-1. These results indicated that treatment with 10 mmol/L of

Fig. 2 Effects of ELA on inflammatory cytokines in HUVEC treated or non-treated with M β CD. HUVEC were treated with 100 μ mol/L oleic acid (9c18:1) and elaidic acid (9t18:1) for 24 h. The lipid rafts were destroyed by 10 mmol/L M β CD for 60 min before ELA supplementation. The fold increases of ICAM-1, VCAM-1 and IL-6 mRNA expressions over the control (PBS alone) condition were calculated. Data are expressed as means \pm SD from three independent experiments. Statistical analysis was carried out using ANOVA. * p < 0.05, ** p < 0.01 when compared to the control group

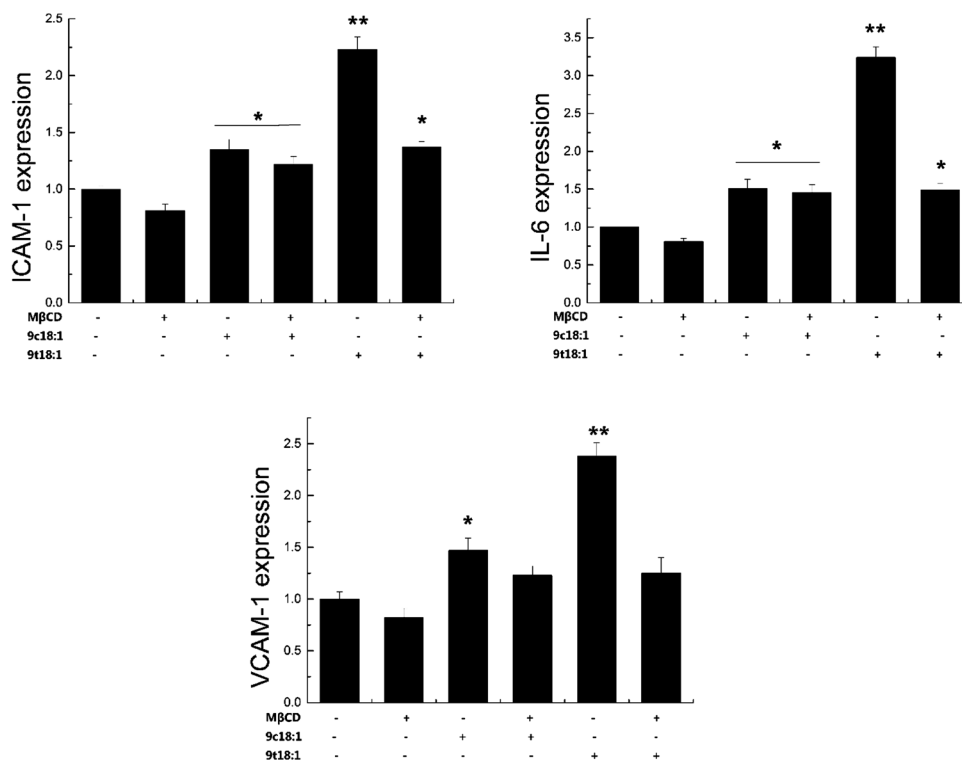


Table 1 The gene sequence of inflammatory cytokines

Gene	Sequence
<i>GADPH forward</i>	5'-CCACCCATGGCAAATTCATGGCA-3'
<i>GADPH reverse</i>	5'-TCTAGACGGCAGGTCAGGTCCACC-3'
<i>ICAM-1 forward</i>	5'-TTCCTACCGTGTACTGGACT-3'
<i>ICAM-1 reverse</i>	5'-GGCAGCGTAGGGTAAGGTTTC-3'
<i>VCAM-1 forward</i>	5'-GGCAGGCTGTAAAAGAATGC-3'
<i>VCAM-1 reverse</i>	5'-TCATGGTCACAGACCCACCT-3'
<i>IL-6 forward</i>	5'-GGTACATCCTCGACGGC-3'
<i>IL-6 reverse</i>	5'-GTGCCTCTTTGCTGCTTTCAC-3'

M β CD within 60 min could interrupt the lipid rafts on cell membrane.

Effects of TFA on the Expression Levels of Inflammation Factors in HUVEC Treated or Non-Treated with M β CD

The expression levels of ICAM-1, VCAM-1 and IL-6 were increased in both OLA and ELA treated cells [(the relative expression levels of ICAM-1 were 1.19 ± 0.32 (treated by OLA) and 2.13 ± 0.41 (ELA), VCAM-1 were 1.48 ± 0.36 (OLA) and 2.32 ± 0.43 (ELA), IL-6 were 1.51 ± 0.24 (OLA) and 3.19 ± 0.46 (ELA), Fig. 2)]. And the gene sequence of inflammatory cytokines was detected (Table 1). However, the increases were more

significant when the cells were treated with ELA than OLA (the expression levels of these adhesion proteins in ELA-treated group were around twice higher than OLA-treated group, Fig. 2). Furthermore, the expression levels of ICAM-1, VCAM-1 and IL-6 were obviously reduced in M β CD + ELA treated group compared with ELA group (the relative expression level of ICAM-1 was decreased from 2.13 ± 0.41 to 1.22 ± 0.36 , VCAM-1 from 2.32 ± 0.43 to 1.12 ± 0.27 , IL-6 from 3.19 ± 0.46 to 1.39 ± 0.32 , p < 0.01, Fig. 2).

According to the results, in ELA and ELA + M β CD groups, when lipid rafts were destroyed, the expressions of these inflammation factors in non-lipid rafts cells would be down-regulated compared with those in normal cells. Therefore, the effect of TFA on inflammation of HUVEC could be inhibited in HUVEC with non-lipid rafts.

Effects of TFA on NF- κ B Activation in HUVEC Treated or Non-Treated with M β CD

In normal cells, both OLA and ELA increased the nuclear translocation level of NF- κ B p65 in comparison with the control group [(the relative level of NF- κ B p65 increased from 1.00 ± 0.12 to 1.83 ± 0.24 (OLA), from 1.00 ± 0.12 to 3.58 ± 0.22 (ELA), respectively, and the significant increases were found in the ELA group (Fig. 3)]. However, the relative level of NF- κ B p65 in ELA + M β CD group decreased from 3.58 ± 0.22 to 1.81 ± 0.22 (p < 0.01,

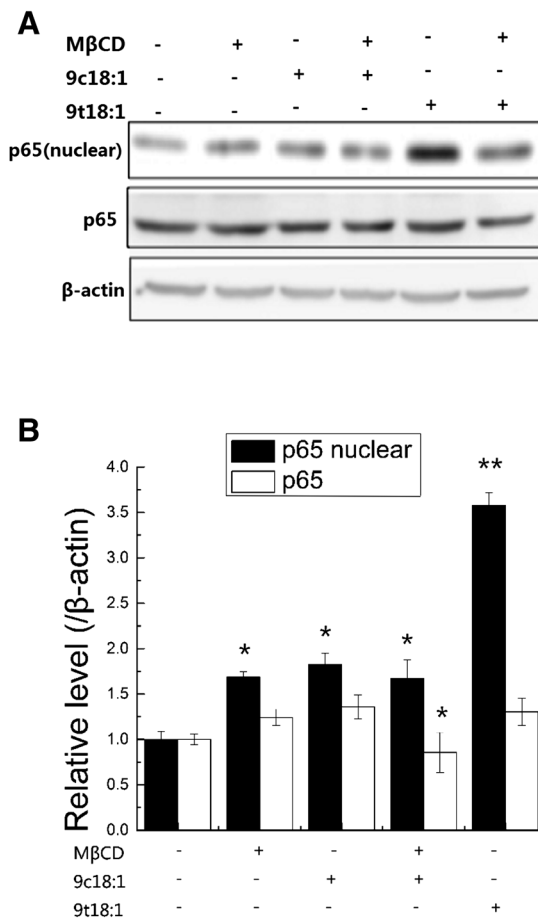


Fig. 3 Effects of TFA on NF- κ B responses in HUVEC treated or non-treated with M β CD. Western blot analysis using nuclear extracts prepared from HUVEC to detect the p65 in nuclear. HUVEC were treated with 100 μ mol/L oleic acid (9c18:1) and elaidic acid (9t18:1) for 24 h. The lipid rafts were destroyed by 10 mmol/L M β CD for 60 min before ELA supplementation (a). The protein levels were quantified by densitometry, and normalize to β -actin levels (b). Data are expressed as means \pm SD from three independent experiments. Statistical analysis was carried out using ANOVA. * p < 0.05, ** p < 0.01 when compared to the control group

Fig. 3), and there was no significant difference between OLA and OLA + M β CD groups (from 1.83 ± 0.24 to 1.67 ± 0.23 , Fig. 3). Thus, the interruption of lipid rafts decreased the nuclear translocation of NF- κ B p65 in HUVEC treated with TFA.

Effects of TFA on ERK1/2 Activation in HUVEC Treated or Non-Treated with M β CD

To determine the effects of ELA and OLA on activation of 1 ERK1/2, HUVEC were incubated in 100 Nmole/L ELA or OLA for 24 h, and the levels of phosphorylated ERK1/2 (p-ERK1/2) were measured. In both OLA and ELA groups, the levels of p-ERK1/2 were increased as compared with the control group

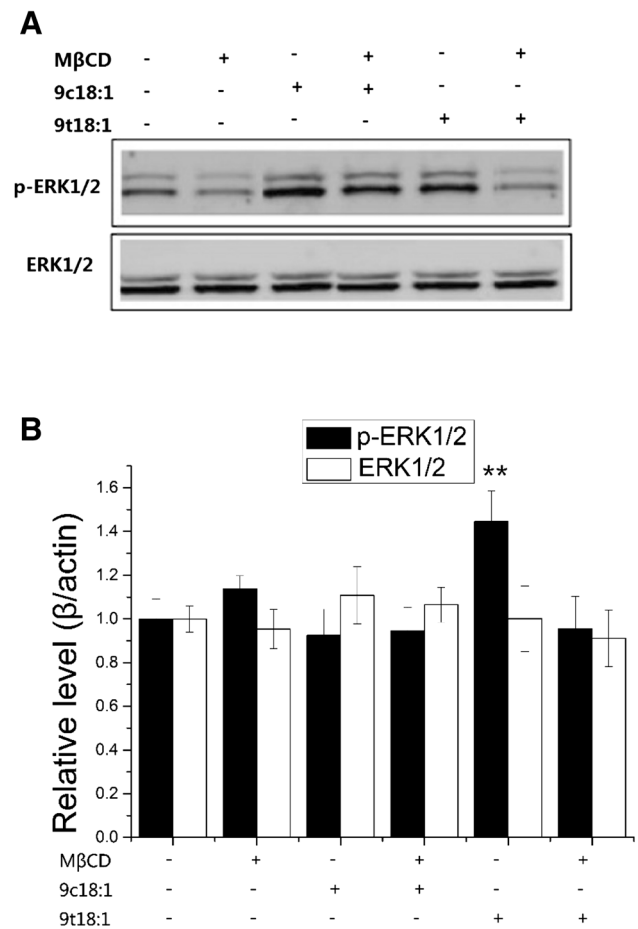


Fig. 4 Effects of TFA on ERK1/2 activation in HUVEC treated or non-treated with M β CD. Human endothelial cells were treated with 100 μ mol/L oleic acid (9c18:1) and elaidic acid (9t18:1) for 24 h. The lipid rafts were destroyed by 10 mmol/L M β CD for 60 min before ELA supplementation. Total ERK1/2 and phosphorylated ERK1/2 (p-ERK1/2) were detected by western blot using specific antibodies (a). The protein levels were quantified by densitometry, and normalize to β -actin levels (b). Data are expressed as means \pm SD from three independent experiments. Statistical analysis was carried out using ANOVA. * p < 0.05, ** p < 0.01 when compared to the control group

(the relative level of p-ERK1/2 was increased from 1.00 ± 0.12 to 1.45 ± 0.24 , p < 0.01, Fig. 4). However, in M β CD + ELA treated group, the level of p-ERK1/2 was significantly decreased as compared with the ELA treated group [1.45 ± 0.24 (M β CD + ELA group), 0.95 ± 0.14 (ELA group), p < 0.01, Fig. 4]. M β CD + OLA treated group showed no obvious changes compared with OLA group [1.11 ± 0.21 (OLA group), 0.91 ± 0.15 (M β CD + OLA group), Fig. 4]. Our results showed that both OLA and ELA activated NF- κ B and ERK1/2 signal pathways to promote the expressions of inflammation factors. However, when lipid rafts were interrupted by M β CD, this activation

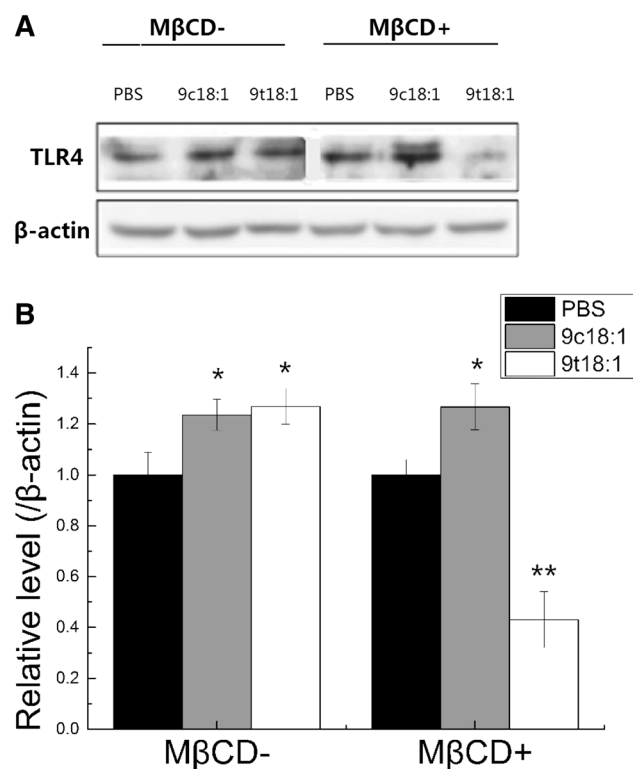


Fig. 5 Effects of TFA on HUVEC TLR4 expression in HUVEC treated or non-treated with MβCD. Human endothelial cells were treated with 100 μmol/L oleic acid (9c18:1) and elaidic acid (9t18:1) for 24 h. The lipid rafts were destroyed by 10 mmol/L MβCD for 60 min before TFA supplementation. The expression of TLR4 was analyzed by western blot (a). The protein levels were quantified by densitometry, and normalized to β-actin levels (b). Data are expressed as means ± SD from three independent experiments. Statistical analysis was carried out using ANOVA. * $p < 0.05$, ** $p < 0.01$ when compared to the PBS-induced group

was restrained. These results indicated that lipid rafts might decrease endothelial ERK1/2 activation after TFA supplementation.

Effects of TFA on TLR4 Expression in HUVEC Treated or Non-Treated with MβCD

The expression levels of TLR-4 in non-lipid rafts cells and normal cells were examined. Both in ELA and OLA treated groups, the expression levels of inflammatory receptor TLR4 were increased as compared to the control group [the expression level of TLR4 increased from 1.00 ± 0.13 to 1.27 ± 0.21 (OLA group), from 1.00 ± 0.15 to 1.27 ± 0.21 (ELA group), Fig. 5]. But after the removal of lipid rafts by MβCD, the expression of inflammatory receptor TLR4 was significantly decreased in the ELA + MβCD group (from 1.27 ± 0.21 to 0.43 ± 0.11 , $p < 0.01$, Fig. 5), with no obvious change in OLA + MβCD group (from 1.27 ± 0.21 to 1.24 ± 0.16 , Fig. 5). Both OLA and ELA increased the

expression of TLR4 protein. However, the expression was decreased in the cells with non-lipid rafts pretreated by ELA compared with the normal cells. When the cells were pretreated with OLA, the damage of lipid rafts showed no obvious effect on the expression of TLR4.

Discussion

Lipid rafts appeared to be in many biological events such as synthetic traffic, cell skeletal reconstruction and signal transduction [28]. In this study, MβCD was used to establish a non-lipid rafts cell model because it interfered with lipid rafts [28]. After being treated with 10 mmol/L of MβCD for 60 min, cells showed no significant changes in both morphology and physiology [29]. The content of cholesterol and expression level of caveolin-1 in cell membrane decreased significantly ($p < 0.05$, Fig. 1). Thus caveolin-1 may be required for the formation of large platforms that control lipid raft-dependent cell uptake or caveolin-1 expression [30]. However, MβCD can inhibit the formation of lipid rafts by eliminating cholesterol [29], which may destroy the caveolae-like membrane, and then down-regulate the expression of caveolin-1. Our results indicated that treatment with 10 mmol/L of MβCD for 60 min interrupted the lipid rafts on cell membrane.

On the other hand, it has been found that caveolin-1 stimulated the expressions of proatherogenic molecules such as CD36 and VCAM-1 [31]. Therefore, the expression levels of VCAM-1, ICAM-1 and IL-6 were measured in both cells with non-lipid rafts and normal cells treated with ELA and OLA, respectively. In this study, compared with OLA group, the expression levels of ICAM-1, VCAM-1 and IL-6 did not change significantly in MβCD + OLA treated cells (Fig. 2). The levels of these adhesion proteins including ICAM-1, VCAM-1 and IL-6 were significantly increased after treated with ELA or OLA. But the expression levels of these adhesion proteins were decreased in MβCD + ELA treated cells ($p < 0.01$, Fig. 2). It was reported that free fatty acid may activate NF-κB pathway via increasing the expression levels of adhesion molecules such as ICAM-1 and VCAM-1, and the releases of pro-inflammatory cytokines such as IL-6 and TNF-α [32]. There was no significant difference between OLA group and OLA + MβCD group. However, in ELA and ELA + MβCD groups, when lipid rafts was destroyed, these inflammation factors in non-lipid rafts cells were down-regulated as compared with those in normal cells. Therefore, lipid rafts may control the expressions of inflammation factors such as ICAM-1, VCAM-1 and IL-6 in TFA-treated cells. In brief, the effects of TFA on the inflammation of HUVEC may be inhibited in non-lipid rafts HUVEC. In this study, both OLA and ELA were

found to up-regulate the nuclear translocation of NF- κ B p65 in HUVEC (Fig. 3). These results indicated that lipid rafts might increase the endothelial NF- κ B activation in HUVEC treated with TFA. When lipid rafts were destroyed in HUVEC pretreated with TFA, the nuclear translocation of NF- κ B p65 was also reduced. Therefore, there might be some special receptors in the lipid rafts which may activate the NF- κ B activation induced by TFA. Based on these results, we inferred that lipid rafts might play a crucial role in NF- κ B activation in HUVEC treated with TFA, and the interruption of lipid rafts may down-regulate the nuclear translocation of NF- κ B p65 in HUVEC treated by TFA.

It has been reported that free fatty acids can induce inflammation by the ERK1/2 pathway [33]. Lipid raft cholesterol was found to regulate the apoptotic cell death in prostate cancer cells through EGFR-mediated Akt and ERK pathways [34]. It was also reported that caveolin-1 functioned as a negative regulator of mitogen-stimulated proliferation and the Ras-p42/44 ERK pathway in a variety of cell types [35]. The overexpression of caveolin-1 inhibited the signaling from ERK1/2 to the nucleus *in vivo* [35]. In this study, the damage of lipid raft was found to down-regulate the expression of caveolin-1 (Fig. 1). Different signaling pathways mediated both overlapping and distinct effects in the activation of both ERK1/2 and MAPK cascades [34], contributing to the stimulation of NF- κ B dependent transcription in endothelial cells. Hence, we inferred that both ERK1/2 and NF- κ B may play important roles in maintaining vascular integrity during a chronic inflammatory response. A previous study showed that chemical disruption of lipid rafts inhibited ERK signaling [34]. Our results showed that both OLA and ELA activated NF- κ B and ERK1/2 signal pathways to promote the expressions of inflammation factors. However, when lipid rafts were interrupted by M β CD, this activation was restrained. Hence, lipid rafts may up-regulate the ERK1/2 activation in HUVEC treated by TFA. The interruption of lipid rafts may decrease the level of phosphorylated ERK1/2 in order to reduce the inflammation of HUVEC. However, whether there was a special receptor in lipid rafts which can activate the pro-inflammation in HUVEC induced by TFA was unknown. Research has shown that the pro-inflammatory mediators trigger the multiple signal transduction pathways through Toll-like receptors (TLR), a family of pattern-recognition receptors expressed in macrophages and other cells [36]. TLR, residing in lipid rafts [18], could recognize pathogen-associated molecular patterns that induce the expression of pro-inflammatory cytokines and initiate pathogen-specific immune responses [36]. Nowadays, multiple lines of evidence suggest that TLR4 may play a crucial role in the onset and maintenance of inflammatory response through binding exogenous and endogenous inflammatory factors and triggering downstream signal transduction pathways [37].

Moreover, the integrity of lipid rafts was important for palmitate-induced TLR4 mediated NF- κ B activation [19, 20, 38]. Accordingly, the inflammatory receptor TLR4 may play a crucial role in inflammation induced by TFA. TLR4 can activate the downstream inflammatory signal pathways like NF- κ B. The nuclear translocation of NF- κ B p65 and phosphorylation of ERK1/2 may increase the expression levels of inflammatory cytokines, and then promote the inflammation. In a nutshell, the results showed that lipid rafts might up-regulate TLR4 expression in HUVEC treated with TFA. And the damage of lipid rafts would decrease the TLR4 expression to reduce the inflammation of HUVEC. It is possible that TFA may directly activate TLR4 or indirectly alter the function of lipid rafts and provide a platform for interaction between TLR4 and TFA. Therefore, the interruption of lipid rafts can decrease the expression of TLR4 in the TFA treated cells.

Conclusion

In conclusion, a possible molecular mechanism involved in 9 α :18:1-induced inflammation in HUVEC was proposed. Both OLA and ELA may stimulate HUVEC inflammation, but ELA may stimulate this inflammation through lipid rafts. Lipid rafts could play an important role in 9 α :18:1-induced inflammation. TLR4 may act as a special protein receptor in lipid rafts and then started the inflammation program after 9 α :18:1 stimulated HUVEC. Then NF- κ B p65 and pERK1/2 were activated when the signals from the inflammation receptor were received. The high expression of p65 and pERK1/2 could increase the expression levels of adhesion proteins including ICAM-1, VCAM-1 and IL-6. When lipid rafts were interrupted by M β CD, the expression levels of adhesion proteins in the TFA treated cells were reduced. ELA can stimulate the inflammatory receptor TLR4, which can activate the inflammatory signal pathways. When lipid rafts were interrupted by M β CD, the TLR4 expression was decreased significantly, which may block the inflammatory signal pathway and down-regulate the expressions of inflammatory cytokines. The study has provided mechanistic insights into the role of lipid rafts in mediating endothelial inflammation by TFA. The damage of lipid rafts may inhibit the inflammation of HUVEC after TFA supplementation. In other words, lipid rafts may provide a platform for a specific receptor protein (TLR4) for TFA to activate the pro-inflammation in cell membranes.

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Compliance with ethical standards

Conflict of interest The authors have declared that there are no conflicts of interest.

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