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Linoleic acid increases monocyte chemotaxis and adhesion to human aortic endothelial cells through protein kinase C- and cyclooxygenase-2-dependent mechanisms☆

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Abstract

The effects of polyunsaturated n-6 linoleic acid on monocyte–endothelial interactions were investigated with particular emphasis on the expression of platelet/endothelial cell adhesion molecule (PECAM)-1 and the role of protein kinase C (PKC) and cyclooxygenase-2 (COX-2). As a diet rich in polyunsaturated fatty acids may favour atherosclerosis in hyperglycaemia, this study was performed in both normal and high-glucose media using human aortic endothelial cells (HAEC). The HAEC were preincubated with normal (5 mM) or high (25 mM) D-glucose for 3 days before addition of fatty acids (0.2 mM) for 3 days. Linoleic acid enhanced PECAM-1 expression independently of tumor necrosis factor (TNF)-α and significantly increased TNF-α-induced monocyte adhesion to HAEC in comparison to the monounsaturated n-9 oleic acid. Chronic glucose treatment (25 mM, 6 days) did not modify the TNF-α-induced or fatty acid-induced changes in monocyte binding. The increase in monocyte binding was accompanied by a significant increase in E-selectin and vascular cell adhesion molecule (VCAM)-1 expression and could be abrogated by an interleukin (IL)-8 neutralising antibody and by the PKC and COX inhibitors. Inhibition of PKC-δ reduced VCAM-1 expression regardless of experimental condition and was accompanied by a significant decrease in monocyte binding. Conditioned medium from linoleic acid-treated HAEC grown in normal glucose conditions significantly increased THP-1 chemotaxis. These results suggest that linoleic acid-induced changes in monocyte chemotaxis and subsequent binding are not solely mediated by changes in adhesion molecule expression but may be due to secreted factors such as IL-8, monocyte chemoattractant protein-1 or prostaglandins (PGs) such as PGE_2, as IL-8 neutralisation and COX-2 inhibition reduced monocyte binding without changes in adhesion molecule expression.

Keywords: Endothelial cells; Fatty acids; Adhesion molecules; Monocyte adhesion; Monocyte chemotaxis; Inflammation

1. Introduction

Numerous studies have demonstrated the relationship between dietary intake of fatty acids and development of cardiovascular disease [1–4]. Dietary fatty acids differentially modulate the progression of atherosclerosis through anti- or proinflammatory mechanisms. The polyunsaturated n-6 linoleic acid (18:2) is the major dietary fatty acid in the Western diet and is considered proatherogenic in comparison to the monounsaturated n-9 oleic acid (18:1), as it promotes endothelial dysfunction, increases atherosclerosis and is associated with hyperinsulinaemia [5].

Atherosclerosis is an inflammatory process, initiated by endothelial dysfunction [6]. Recruitment of monocytes to the endothelium is one of the earliest events in the formation of an atherosclerotic lesion, and it is well established that chemokines [interleukin (IL)-8, monocyte chemoattractant protein (MCP)-1], cytokines [tumor necrosis factor (TNF)-α] and adhesion molecules [E-selectin, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1] play an important role in this process [7,8]. Molecules such as platelet/endothelial cell adhesion molecule (PECAM)-1 contribute to monocyte transmigration through the endothelium to the subendothelial space where they differentiate into macrophages [9]. The expression of these proatherogenic genes is regulated by nuclear factor (NF)-κB, an oxidative stress-induced transcription factor [10]. Circulating free dietary fatty acids, derived from lipoprotein lipase-mediated hydrolysis of triglyceride-rich lipoproteins, and accumulated lipids in the subendothelial space can modulate the endothelial inflammatory response through regulation of gene expression via NF-κB or various cell signalling pathways including phosphatidylinositol 3-kinase (PI3K), p38 mitogen-activated protein kinase (p38 MAPK) [11] and enzyme-mediated pathways such as protein kinase C (PKC) and cyclooxygenase (COX) [12,13]. The effects of fatty acids on cell signalling mechanisms differ among different types of cells because the pathways by which they are metabolised are dependent on the elongase and desaturase profiles of the individual cell type. Endothelial cells lack Δ6 desaturase activity but contain Δ5 and Δ9

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desaturase activities [14], and it has been suggested that defects in Δ5 and Δ6 desaturase activities may contribute to atherogenesis [15].

Circulating free fatty acids and high glucose contribute to atherogenesis in vascular cells via activation of PKC resulting in an increase of endothelial permeability, cytokine activation and leukocyte adhesion [16]. In addition, the proinflammatory enzyme COX-2 may also be involved in the pathogenesis of atherosclerosis, as it is expressed in atherosclerotic lesions and increased after vascular injury [17]. Cyclooxygenase-2 plays a role in lipid metabolism, catalyzing the rate-limiting step of prostaglandin (PG) synthesis from fatty acids, and has been shown to be responsible for the oxidative metabolism of linoleic acid in endothelial cells [18]. Up-regulation of COX-2 has also been associated with the chronic low levels of inflammation in diabetes [19], where an alteration of fatty acid metabolism has been described.

The aim of this study was to investigate the effect of linoleic acid on monocyte–endothelium interactions and the expression of PECAM-1 and to evaluate the contribution of PKC and COX-2 signalling. As diets rich in polyunsaturated fatty acid (PUFA) may favour atherosclerosis in hyperglycaemia, this study was performed in both normal and high-glucose media using (the physiologically relevant) human aortic endothelial cells (HAEC). The impact of linoleic acid vs. oleic acid was assessed in *in vitro* conditions simulating some of the aspects of chronic inflammation *in vivo*.

2. Materials and methods

2.1. Materials

Human aortic endothelial cells and endothelial cell growth medium MV were purchased from Promocell (Heidelberg, Germany). THP-1 cells were obtained from European Collection of Cell Cultures (ECACC) (Salisbury, UK). RPMI medium, Glutamax and nonessential amino acids were obtained from Invitrogen (Paisley, UK). Antibiotics, linoleic acid, oleic acid, fatty acid-free bovine serum albumin (BSA), D-glucose, indomethacin and calphostin C were supplied by Sigma (Poole, UK). Tumor necrosis factor-α, IL-8 neutralising antibody and monoclonal ICAM-1 antibody were obtained from R&D Systems (Abingdon, UK). Rottlerin was purchased from Calbiochem (Merck Chemicals Ltd., Nottingham, UK). LY397196 was a kind gift from Eli Lilly. Calcein AM, biotinylated goat anti-mouse immunoglobulin (Ig) G and streptavidin horseradish peroxidase (HRP)-conjugated IgG were supplied by Invitrogen, and 3,3′,5,5′-tetramethylbenzidine, by Chemicon (Millipore UK Ltd., Watford, UK). Primary antibodies against E-selectin, PECAM-1, α-tubulin as well as the HRP-conjugated secondary antibody were purchased from Abcam (Cambridge, UK), and monoclonal VCAM-1 antibody and COX-2 antibody were obtained from Chemicon and Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), respectively. Precast gels and enhanced chemiluminescence detection kit were supplied by Pierce (Thermo Scientific, Rockford, IL, USA).

2.2. Cell culture

Human aortic endothelial cells were grown in 5% serum endothelial cell growth medium MV supplemented with antibiotics (penicillin G 60 μg/ml, streptomycin 100 μg/ml) for a total of 6 days. All experiments were performed using cells of Passage 2–5 and endothelial monolayers at 80% confluence. Chronic glucose and fatty acid conditions were established by preincubating HAEC with normal (5 mM) or high (25 mM) D-glucose for 3 days prior to addition of fatty acids (0.2 mM) for 3 days. Fatty acids, monounsaturated fatty acid (MUFA) oleic acid (18:1) and PUFA linoleic acid (18:2) (0.2 mM), were added using defatted BSA as a carrier in a ratio of 6:1 [20]. Tumor necrosis factor-α stimulation was performed prior to extraction (10 ng/ml, 4–12 h). The COX-2 inhibitor indomethacin (10 μM, 4 h); the PKC inhibitors calphostin C (50 nM, 18 h), LY397196 (30 nM, 5 h) and rottlerin (1 μM, 4 h); and an IL-8 neutralising antibody (20 μg/ml, 4 h) were also used.

2.3. Fatty acid profile

Fatty acid incorporation into HAEC phospholipids after experimental treatment was analysed by gas chromatography as described previously [20].

2.4. Monocyte adhesion to endothelial cells

The number of fluorescently labelled THP-1 cells (2×10^6 cells) bound to HAEC monolayers was quantified by fluorescence microscopy (Olympus X51) using Labworks 4.5 image acquisition and analysis software (UVP). For details, see supplementary methods.

2.5. Cell surface expression of adhesion molecules

The cell surface expression of the adhesion molecules, E-selectin, VCAM-1 and ICAM-1, was measured in fixed cells by enzyme-linked immunosorbent assay (ELISA) as described in the supplementary methods.

2.6. Chemokine production

Interleukin-8 and MCP-1 production was measured in cell culture supernatants using ELISA methods according to the manufacturer's instructions (R&D Systems).

2.7. COX-2 and PECAM expression by Western blotting

Protein expressions of COX-2 and PECAM were quantified in HAEC by Western blotting using monoclonal antibodies to COX-2 (1:500, overnight), PECAM (1:1000, 1 h) and α-tubulin (1:500, 1 h). For details, see supplementary methods.

2.8. Monocyte chemotaxis

THP-1 chemotaxis to conditioned medium from fatty acid-treated HAEC was assessed using the Neuro Probe MBC96 chamber (Gaithersburg, MD, USA) with polycarbonate filters (8 μM) as described in the supplementary methods.

2.9. Statistics

All results were subjected to one-way analysis of variance with Bonferroni post hoc multicomparison statistical analysis using GraphPad Prism 4 and Microsoft Excel. At least three independent experiments were performed for each condition, and data are expressed as means±S.E.M. unless otherwise stated ($P<.05$).

3. Results

3.1. Fatty acid profile

PUFA linoleic acid and MUFA oleic acid incorporated significantly into endothelial cells, changing the fatty acid profile after 72 h of incubation in both normal and high-glucose conditions (Table 1). PUFA treatment increased the total linoleic acid content by more than 30%, while MUFA treatment increased the oleic acid content by 25% in comparison to the control. Total SFA and ω3-PUFA were not modified after treatment (Table 1).

3.2. Monocyte binding

Tumor necrosis factor-α stimulation significantly increased THP-1 binding to HAEC and was differentially modulated by fatty acid treatment (Fig. 1A). Addition of the PUFA linoleic acid significantly increased TNF-α-induced monocyte adhesion (Fig. 1A), with the highest number of monocytes binding in high-glucose-treated HAEC. In comparison, the MUFA oleic acid significantly reduced TNF-α-induced monocyte binding in high-glucose conditions (Fig. 1A).

Specific inhibitors were used to investigate the potential signalling mechanisms involved in linoleic acid-induced monocyte binding to TNF-α-stimulated endothelial cells. As chronic high-glucose treatment (6 days) did not significantly modify linoleic acid-induced monocyte vs. chronic normal glucose binding, only the data for linoleic acid treatment in normal glucose conditions are shown (Fig. 1B). Addition of the general PKC inhibitor calphostin C (50 nM, 18 h) and the COX-2 inhibitor indomethacin abrogated the linoleic acid-induced increase in monocyte binding (Fig. 1B). The use of isoform-specific inhibitors demonstrated that PKC-δ may be involved in linoleic acid-induced monocyte binding as rottlerin (1 μM, 4 h) blocked linoleic acid-induced monocyte binding. The PKC-βII inhibitor LY397196 (30 nM, 5 h) significantly ($P<.05$) reduced monocyte binding in all chronic high-glucose conditions tested (114±4% vs. 67±3%, normal glucose vs. high glucose; 145±14% vs. 86±4%, normal glucose+linoleic acid vs. high glucose+linoleic acid).

Table 1
Fatty acid profile in HAEC was analysed by gas chromatography

	Normal glucose			High glucose		
	C	OA	LA	C	OA	LA
Saturates						
MA (14:0)	4.25±0.45	2.3±1.00	1.90±0.10	4.45±0.95	2.25±0.95	2.55±0.15
PA (16:0)	34.25±6.45	18.20±3.00	20.55±0.25	27.40±1.10	21.70±3.70	24.00±0.90
SA (18:0)	16.00±6.50	5.60±0.60	6.80±0.60	9.20±1.70	8.55±0.45	9.75±1.35
Total SFA	54.50±13.40	26.10±3.40	29.25±0.25	41.05±0.35	32.50±5.10	36.30±2.10
Monosaturates						
PLA (16:1ω6)	8.50±0.00	2.55±0.35 *	1.80±0.30 *	8.70±0.50	2.20±0.20 #	2.75±1.95 #
OA (18:1ω9)	30.30±1.80	55.20±4.60 *	7.60±0.00	26.90±0.80	52.50±4.30 #	15.40±5.90
Total MUFA	38.80±1.80	57.75±4.25	9.40±0.30 *	35.60±0.30	54.70±4.10	18.15±7.85 #
ω6-Polysaturates						
LA (18:2ω6)	1.15±1.15	1.75±0.75	47.55±0.05 *	2.30±0.20	1.70±0.70	29.70±10.80 #
γ-LA (18:3ω6)	0.15±0.15	1.00±0.60	1.30±1.00	0.80±0.30	0.65±0.35	1.05±1.05
Dh-γ-LA (20:3ω6)	1.60±0.00	1.70±1.10	2.10±0.70	1.70±0.00	1.60±0.90	1.85±0.25
AA (20:4ω6)	8.20±0.00	4.70±0.40 *	4.65±0.05 *	8.20±0.10	3.75±0.65 #	5.35±0.75 #
DTA (22:4ω6)	–	1.25±0.35	0.95±0.95	0.50±0.50	0.70±0.70	1.60±0.00
Total ω6-PUFA	11.10±1.30	9.20±0.60	56.70±1.10 *	13.25±0.15	7.85±0.05	39.90±10.90 #
ω3-Polysaturates						
α-LA (18:3ω3)	–	–	0.70±0.70	0.40±0.40	–	–
MoA (18:4ω3)	2.50±0.00	0.95±0.35	0.65±0.65	2.70± 0.00	1.40±0.60	0.20±0.20 #
EPA (20:5ω3)	0.80±0.80	0.20±0.20	1.10±1.10	1.55±1.50	0.20±0.20	0.40±0.40
DPA (22:5ω3)	4.00±0.00	2.45±0.35	1.95±0.05	3.60±0.20	2.50± 0.00	2.40±0.60 #
DHA (22:6ω3)	2.15±2.15	2.75±0.05	1.05±0.05	2.00±2.00	1.85±0.45	1.75±0.75
Total ω3-PUFA	9.90±0.00	6.95±0.35	5.10±0.90	8.00±2.10	5.05±0.95	5.75±0.95

Cells were incubated in normal (5 mM) or high (25 mM) D-glucose for 3 days and then treated for 3 days with linoleic acid or oleic acid using BSA as carrier. Results are expressed as % of total fatty acid content. C: BSA control; MA: myristic acid; PA: palmitic acid; SA: stearic acid; PLA: palmitoleic acid; OA: oleic acid; LA: linoleic acid; γ-LA: γ-linoleic acid; Dh-γ-LA: dihomo-γ-linoleate; AA: arachidonic acid; DTA: docosatetraenoic acid; α-LA: α-linoleic acid; MoA: moroctic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoate.

* $P<.05$ vs. control low glucose.

\# $P<.05$ vs. control high glucose.

Chemokines such as IL-8 may play a role in monocyte binding. Linoleic acid increased IL-8 production from HAEC (3.9±0.6 vs. 1.8±0.6 ng/ml, $P<.05$, $n=3$). Furthermore, the use of an IL-8 neutralising antibody (20 μg/ml, 4 h) reduced linoleic acid-induced monocyte binding below control levels (Fig. 1B).

3.3. COX-2 expression

Linoleic acid increased COX-2 expression in TNF-α-stimulated HAEC as detected by Western blotting (Fig. 2A). Inhibition of PKC by the general PKC inhibitor calphostin C significantly reduced COX-2 expression in all conditions tested.

3.4. Adhesion molecule expression

A critical receptor implicated in leukocyte transmigration is PECAM-1 (CD31), which is expressed on the surface of platelets, leukocytes and endothelial cells, particularly at intercellular junctions [9]. Platelet/endothelial cell adhesion molecule-1 may be phosphorylated on serine residues by PKC and has been implicated in glucose-induced transmigration of monocytes through human umbilical vein endothelial cells (HUVEC) monolayers [21]. In normal glucose conditions, linoleic acid significantly increased PECAM-1 levels with respect to control cells (Fig. 2B).

Similar to monocyte binding results, chronic glucose treatment (25 mM, 6 days) did not significantly modify the endothelial cell surface expression of adhesion molecules (data not shown). However, changes in monocyte adhesion to TNF-α-stimulated HAEC monolayers were accompanied by increased mRNA ($P<.05$) and HAEC surface expression ($P<.0001$) of E-selectin (+100%), ICAM-1 (+200%) and VCAM-1 (+250%). Treatment with linoleic acid in combination with TNF-α further increased endothelial E-selectin and VCAM-1 cell surface expression (Table 2). Protein kinase C-δ inhibition (rottlerin; 1 μM) significantly reduced VCAM-1 expression but had no effect on E-selectin and ICAM-1 cell surface expression (Table 2).

3.5. Monocyte chemotaxis

To further investigate linoleic acid-induced changes to monocyte–endothelial interactions, monocyte (THP-1) chemotaxis to conditioned normal glucose medium from linoleic acid-treated HAEC was quantified using a Neuro Probe chemotaxis chamber (Fig. 3). Medium from linoleic acid-treated HAEC significantly increased monocyte chemotaxis in comparison to control.

4. Discussion

Dietary fatty acids can differently modulate the progression of atherosclerosis, promoting pro- or antiatherogenic effects. While MUFAs, such as n-9 oleic acid, appear to be antiatherogenic, inhibiting or down-regulating endothelial cell activation and subsequent inflammatory response [22], the polyunsaturated n-6 linoleic acid has been ascribed proatherogenic properties, inducing endothelial dysfunction [5]. In this study, we report that linoleic acid modulates the interaction between HAEC and monocytes, resulting in increased monocyte chemotaxis and subsequent binding through increased molecule adhesion expression and IL-8 secretion, via PKC- and COX-2-dependent mechanisms.

This study confirms the proinflammatory effects of linoleic acid in HAEC and is in agreement with published studies performed on cells from different species [11,23] and/or vascular beds [24]. In addition, this is the first reported effect of linoleic acid on PECAM-1 expression in HAEC. Here, we show that linoleic acid enhances PECAM-1 expression independently of TNF-α. It is noteworthy that linoleic acid enhances further E-selectin and VCAM-1 expression and monocyte binding induced by TNF-α. It has recently been reported that the linoleic acid modulation of the TNF-α-induced

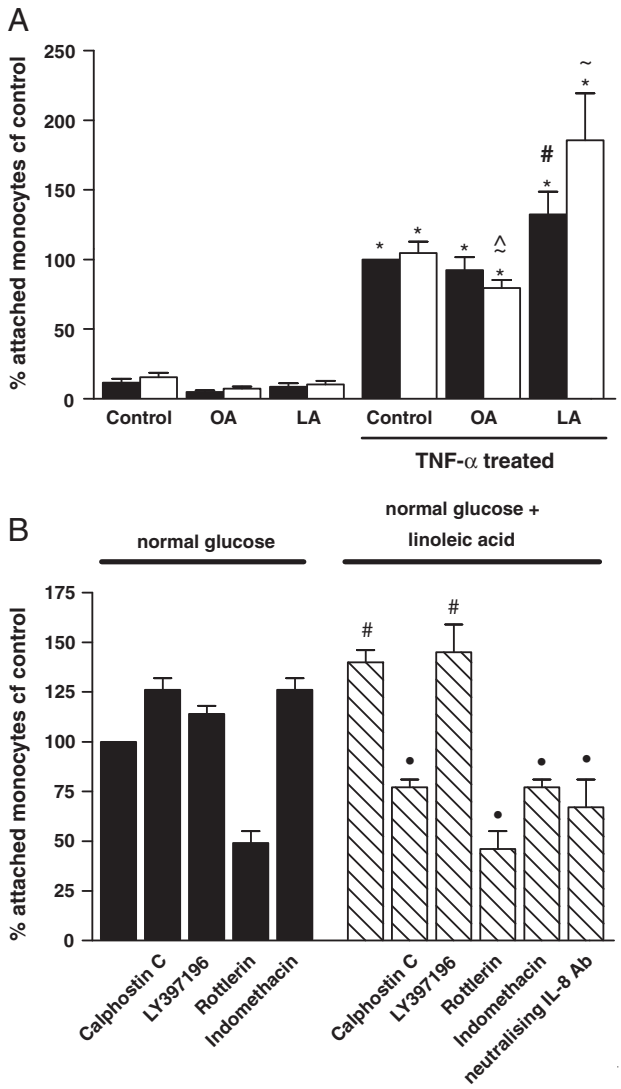

Fig. 1. Effects of fatty acids and inhibitors on monocyte binding to glucose-treated HAEC monolayers. (A) HAEC were preincubated with normal (■ 5 mM) or high (□ 25 mM) D-glucose for 3 days before addition of linoleic (LA) or oleic (OA) acids (0.2 mM) using fatty acid-free BSA as carrier for 3 days. TNF-α stimulation (10 ng/ml, 4 h) was performed at the end of the experimental period. (B) HAEC were preincubated with normal (5 mM) D-glucose for 3 days before the addition of linoleic acid in fatty acid-free BSA (0.2 mM) for 3 days. TNF-α stimulation (10 ng/ml, 4 h) and the addition of the inhibitors [calphostin C (50 nM, 18 h), LY397196 (30 nM, 5 h), rottlerin (1 μM, 4 h), indomethacin (10 μM, 4 h) and IL-8 neutralising antibody (20 μg/ml, 4 h)] were performed at the end of the experimental period. The mean number of attached monocytes is expressed as a percentage of the normal glucose BSA control. *P<.05 vs. nonstimulated, #P<.05 vs. normal glucose BSA control, ˜P<.05 vs. high-glucose BSA control, ^P<.001 vs. high-glucose linoleic acid treated, •P<.05 vs. normal glucose linoleic acid treated. n=4 independent experiments.

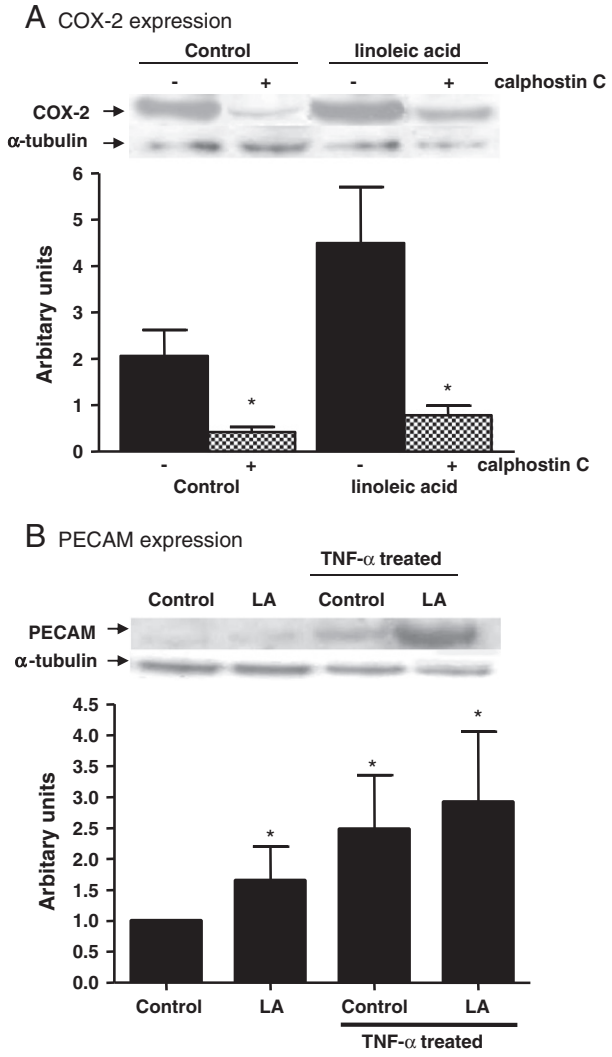

Fig. 2. Linoleic acid (LA) effects on COX-2 and PECAM expression in HAEC. HAEC monolayers were preincubated with normal (5 mM) D-glucose for 3 days before addition of linoleic acid (0.2 mM) using fatty acid-free BSA as carrier. (A) COX-2 expression was quantified in TNF-α-stimulated HAEC by Western blotting. The general PKC inhibitor calphostin C (+, 50 nM, 18 h) was added prior to TNF-α stimulation (10 ng/ml, 4 h). *P<.05 vs. without PKC inhibitor. (B) The effects of linoleic acid (0.2 mM, 3 days) and TNF-α stimulation (10 ng/ml, 4 h) on PECAM expression were quantified by Western blotting. The results are expressed relative to the BSA control. *P<.05 vs. BSA control. n=4 independent experiments.

proinflammatory response in porcine pulmonary artery endothelial cells requires functional caveolae [25], and these membrane domains are also implicated in COX-2 regulation by linoleic acid [26]. Thus, linoleic acid may not only induce proinflammatory events *per se*, but also exacerbate a previous inflammatory status. This is particularly important in diseases such as atherosclerosis or diabetes that are associated with an inflammatory state because the prognosis of such diseases may be impaired by consuming a diet with high PUFA content.

With regard to the effects of glucose, most of the studies to date have been performed using HUVEC and contradictory results have been reported concerning the effects of glucose on expression of adhesion molecules (E-selectin, VCAM-1, ICAM-1). These differences may be caused by different glucose concentrations,

Table 2
Effects of linoleic acid and PKC-δ inhibition on adhesion molecule expression in HAEC

	Normal glucose		Linoleic acid	
	BSA control	Rottlerin		Rottlerin
E-selectin	100	110±3 *	141±4 *	152±3 *
VCAM-1	100	55±3 *	125±4 *	67±8 ˜
ICAM-1	100	92±3 #	104±4	102±9

Confluent HAEC were preincubated with normal (5 mM) D-glucose for 3 days before addition of linoleic acid (0.2 mM) and TNF-α (10 ng/m, 4 h) as described. The PKC-δ inhibitor rottlerin (50 nM, 18 h) was added at the end of the experimental period, and adhesion molecule surface expression was determined in the fixed cells by ELISA. The results are expressed as the mean percentage of the normal glucose BSA control.
* P<.0001 vs. normal glucose BSA control.
# P<.05 vs. normal glucose BSA control.
˜ P<.05 vs. linoleic acid treated. n=10.

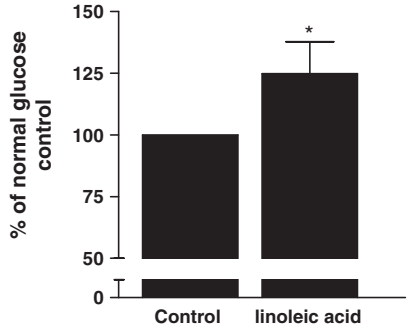

Fig. 3. Effects of glucose and linoleic acid on monocyte chemotaxis. THP-1 migration to conditioned medium from confluent HAEC incubated in glucose (normal; 5 mM glucose) for 3 days before addition of linoleic acid was assessed using a Neuro Probe chemotaxis chamber. Results are expressed as a percentage of the BSA control. *P<.05 vs. control. n=3 independent experiments.

incubation times and cell types used. Where enhanced adhesion molecules were found, cells were incubated for shorter times, imitating an acute rather than a chronic response [27,28]. In this study, 6-day glucose treatment had no effect on the endothelial cell–monocyte interactions, as it did not modify TNF-α-induced adhesion molecule surface expression and monocyte binding. Srinivasan et al. [29] observed increased monocyte binding to glucose-treated HAEC only after 7 days of culture. This was accompanied by an increased production of IL-8 and thought to be mediated by the transcription factor AP-1 and p38 MAPK signalling [30]. Acute rather than chronic effects of glucose have also been reported. Glucose (25 mM, 2 h) promotes transmigration of monocytes through HUVEC monolayers via phosphorylation of PECAM-1 by PKC [21]. Piga et al. [31] showed a transient increase in VCAM-1 expression that peaked at 4 h and returned to basal levels by 12 h. These acute high-glucose effects may be mediated via PKC-βII, as selective inhibition of PKC-βII prevented the high-glucose-induced VCAM-1 expression [32] and significantly reduced monocyte binding in all high-glucose conditions tested in our study. Taken together, these results would suggest a biphasic response to glucose mediated via PKC activation. Indeed, Quagliaro et al. [33] demonstrated that intermittent high glucose (alternating 5 and 20 mM every 24 h for 14 days) induced a greater expression of adhesion molecules than stable high glucose through the activation of PKC but only after 7 days of culture.

The general PKC inhibitor calphostin C demonstrated the potential role of PKC signalling in linoleic acid-induced effects on HAEC. This was further investigated using isoform-specific inhibitors. Inhibition of PKC-δ reduced VCAM-1 expression regardless of experimental condition and was accompanied by a significant decrease in TNF-α-induced monocyte binding, further confirming the role of VCAM-1 in monocyte–endothelial interactions. These results suggest that linoleic acid-induced changes in monocyte binding are not solely mediated by changes in adhesion molecule expression but may be due to secreted factors such as IL-8, MCP-1 or PGs such as PGE_2, as IL-8 neutralisation and COX-2 inhibition reduced monocyte binding.

Furthermore, it is possible that PKC is acting upstream of COX-2, as the use of the general PKC inhibitor calphostin C reduced linoleic acid-induced COX-2 expression in HAEC. The regulation of COX-2 is complex and can be controlled via activation of various transcription factors including NF-κB and CREB, which can be activated via PKC, resulting in production of PGs. One such PG, 8-iso-PGE_2, has been shown to stimulate HUVEC to bind monocytes but not through the action of NF-κB, VCAM-1 or E-selectin [34]. In summary, linoleic acid may exert its proinflammatory and proatherogenic effects through increased production of reactive oxygen species, activation of transcription factors (NF-κB, AP-1, CREB) and numerous signalling pathways (PI3K, p38 MAPK, PKC, COX-2), which all contribute to endothelial dysfunction.

In conclusion, this study has demonstrated that linoleic acid modulates the interaction between HAEC and THP-1 monocytes, resulting in increased monocyte migration and subsequent adhesion of THP-1 monocytes to human aortic endothelial monolayers through increased IL-8 production, PECAM-1 expression and TNF-α-induced E-selectin and VCAM-1 expression. These changes may be mediated via PKC- and COX-2-dependent mechanisms.

Supplementary materials related to this article can be found online at doi:10.1016/j.jnutbio.2011.03.020.

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