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SHORT COMMUNICATION

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# Effect of fatty acids on expression of endothelial leukocyte adhesion molecules

**Summary** *Background* With respect to linoleic acid both beneficial and proatherogenic effects have been described. However, the effect on expression of cell adhesion molecules on human coronary artery endothelial cells (HCAEC) is not yet established. The aim of the experiments was to evaluate the influence of linoleic acid in comparison with palmitic acid regarding the cytokine-induced expression of endothelial leukocyte adhesion molecules (intercellular cell adhesion molecule-1 ICAM-1, vascular cell adhesion molecule-1 VCAM-1, E-selectin). *Methods* HCAEC were cultured in microvascular endothelial cell growth medium. In the experiments, the cells were preincubated with linoleic acid and palmitic acid, respectively (10 µmol/l, 2 days) or under control conditions, after which interleukin-1α (IL-1α, 10 ng/ml in the test medium) was added for 1 day. The monoclonal antibodies used were fluorescein isothiocyanate (FITC)-labeled anti-ICAM-1, FITC-labeled anti-VCAM-1, and FITC-labeled anti-E-selectin. Expression was analyzed by flow cytofluorimetry. Next, to examine the effects of fatty acids on adhesion of monocytes to endothelial cells, adhesion experiments with the monocytic U 937 cell line were performed. *Results and conclusions* IL-1α increased ICAM-1, VCAM-1, and E-selectin expression compared to controls. Incubation with IL-1α together with linoleic acid reduced the expression of ICAM-1 and VCAM-1 in contrast to palmitic acid. Furthermore, in the presence of linoleic acid a tendency of diminished adhesion of monocytes is seen.

The results indicate that a reduced expression of cell adhesion molecules may be relevant to the antiatherogenic effects of linoleic acid. This is in contrast to the properties of palmitic acid.

**Key words** endothelial cell – adhesion molecule – fatty acids – interleukin-1α

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## Introduction

The understanding of development of atherosclerosis has evolved from epidemiologic identification of risk factors for atherosclerosis to an increased understanding of the molecular basis of the processes. Dietary fatty acids affect plasma lipoproteins and thus are linked to atherosclerosis. However, the effects of fatty acids on vascular functions are complex and also involve responses in vascular endothelial cells. With respect to linoleic acid (C18:2n-6), both beneficial and proatherogenic effects have been described. For example, linoleic acid suppresses expression of fatty acid synthase mRNA and other lipogenic enzymes [1]. A high dietary intake of linoleic acid seems to be associated with longevity [2]. On the other hand, several data suggest that linoleic acid appears to be effective in activating vascular endothelial cells and in contributing to an inflammatory response [3]. Furthermore, a diet rich in linoeic acid seems to lead to increased oxidative stress, which may predispose to endothelial dysfunction [4].

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Adhesion of leukocytes (monocytes) to endothelial cells and transendothelial migration of monocytes are early steps in both inflammation and in the atherosclerotic process. Binding of circulating monocytes to vascular endothelial cells and their subsequent transendothelial migration into the subendothelial space are mediated by inducible cell adhesion molecules (CAMs), expressed on the surface of endothelial cells. The surface expression of CAMs appears to be an important endothelial response to various risk factors for atherosclerosis [5].

Furthermore, elevated plasma levels of circulating CAMs were found in patients with cardiovascular diseases and several risk factors for atherosclerosis [6–8]. Interestingly, recent studies showed that both monounsaturated and polyunsaturated fatty acids may inhibit expression of endothelial leukocyte adhesion molecules [9–13]. Thus, unsaturated fatty acids may contribute to the prevention of cardiovascular diseases through modulation of CAM gene expression.

Experiments with respect to the CAM expression on endothelial cells were carried out with human saphenous vein endothelial cells, human coronary artery endothelial cells and human umbilical vein endothelial cells. However, although endothelial cells have many functional characteristics in common, endothelial cells of various origin may differ in functionality. Therefore, in the present experiments normal primary human coronary artery endothelial cells (HCAEC) were used. The aim of the present study was to show the possible effect of linoleic acid in modulating endothelial CAM expression in comparison with palmitic acid, an abundant saturated fatty acid esterified in phospholipids of cell membranes. Using HCAEC activated by cytokines *in vitro*, the effects of these fatty acids on the expression of intercellular cell adhesion molecule-1 (ICAM-1, CD54), vascular cell adhesion molecule-1 (VCAM-1, CD106), and endothelial leukocyte adhesion molecule-1 (E-selectin, CD62E) were assessed. Moreover, modifications induced by fatty acids on the adhesion of monocytes to endothelial cells were studied.

## Materials and methods

### Endothelial cell cultures

The cryopreserved human coronary artery endothelial cells (HCAEC) were purchased from Clonetics Cell Systems (San Diego, CA, USA). The cells were thawed and washed with phosphate buffer saline (PBS), after centrifugation the cells were resuspended and diluted with endothelial growth media to a concentration of 200,000 cells per ml and seeded in a concentration of 5,000 cells per $cm^2$ in flasks. The cells were maintained in Microvascular Endothelial Growth Medium-2, obtained from Microvascular Endothelial Basal Medium-2 (Clonetics Cell Systems).

### Incubation with linoleic and palmitic acid

The subconfluent cultured cells were preincubated with linoleic acid or palmitic acid (Na salts, Sigma-Aldrich, Deisenhofen, Germany) in the concentrations 10 µmol per liter of the culture media. The time of incubation with the fatty acids was 2 days.

The subconfluent cultured cells of the second to fourth passage were harvested non-enzymatically with Cell Dissociation Solution (Sigma-Aldrich). The ratio of the number of vital and dead cells in the suspension was obtained by staining the cells with trypan blue dye and counting by Neubauer hemacytometer.

### Measurement of ICAM-1, VCAM-1, and E-selectin by flow cytometric analysis

The subconfluent monolayers of HCAEC were exposed to interleukin-1α (IL-1α, 10 ng per ml medium, Clonetics Cell Systems) for 1 day. After the indicated incubation time the cells were harvested and incubated with the fluorescent ICAM-1 and VCAM-1 antibodies (FITC-labeled anti-ICAM-1, FITC-labeled anti-VCAM-1, R & D Systems Europe Ltd., Abingdon, GB) or E-selectin antibody (FITC-labeled anti-E-selectin, Calbiochem, Bad Soden, Germany) for 45 min at 4 °C. After this incubation the cells were washed and fixed with PBS containing 4% paraformaldehyde at room temperature for 15 min. The surface expression of the ICAM-1, VCAM-1, and E-selectin was measured by using the fluorescence activated cell sorting (FACS) analyzer equipped with a single 488-nm argon laser (Becton Dickinson, San Jose, CA). A minimum of 5,000 cells per sample was analyzed.

### Monocyte adhesion assay

The monocytic U 937 cell line (DSMZ GmbH, Braunschweig, Germany) was used in adhesion experiments. The subconfluent cultured HCAEC were preincubated with linoleic or palmitic acid as described above for 2 days, and with IL-1α for 1 day. After the incubations the wells containing the HCAEC were rinsed two times with PBS, and 5 x $10^5$ U937 cells in growth medium (RPMI 1640 with 10% heat inactivated fetal calf serum) were added to each well. After 1 h, the nonadherent monocytic cells were rinsed off with microvascular endothelial growth medium-2. The number of attached monocytic cells was counted in 10 microscopic fields defined by a color video camera 3CCD image [14].

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## Results

Flow cytometric analysis of HCAEC confirmed decreased IL-1α stimulated expression of CAMs by linoleic acid. CAM stimulation was studied at different IL-1α concentrations. A concentration-dependent stimulation of expression of ICAM-1, VCAM-1, and E-selectin by IL-1α was observed with an optimal concentration of 10 ng/ml in the test medium. The concentrations of linoleic acid and palmitic acid used in these experiments lie within the range of those in blood plasma under conditions of average nutrition.

The effects of IL-1α, linoleic acid plus IL-1α, and palmitic acid on the expression of ICAM-, VCAM-1, and E-selectin by HCAEC are shown in Table 1. IL-1α increases ICAM-1, VCAM-1 and E-selectin expression in comparison to controls. Incubation of HCAEC together with linoleic acid reduces the expression of ICAM-1, and VCAM-1 in contrast to palmitic acid. Preincubation of endothelial cells with linoleic acid did not significantly inhibit the cytokine-induced expression of E-selectin.

Moreover, in the present study the capacity of linoleic acid to modify the adhesion of monocytoid cells to HCAEC was assayed. In the presence of linoleic acid a tendency of diminished adhesion in comparison with IL-1α-stimulated endothelial cells is seen (U 937 cell adhesion $108 \pm 62$ n/mm$^2$). Palmitic acid is without effect.

## Discussion

The results of the present study indicate a decreased cytokine-stimulated ICAM-1 and VCAM-1 expression on primary coronary artery endothelial cells in the presence of linoleic acid and a tendency of reduced adherence of monocytes. Since monocyte infiltration plays a major role in the pathogenesis of atherosclerosis, this observation may be relevant to the antiatherogenic properties of linoleic acid.

A crucial step for extravasation of monocytes is binding to endothelial CAMs. Rolling, adhesion and transmigration of monocytes are mediated by these molecules expressed on the cell surface. E-selectin mediates rolling and activation of monocytes. The selectin-ligand interactions are characterized by rapid bond formation to promote adherence, and fast dissociation to facilitate rolling. Thus, selectins mediate the first step in monocyte adhesion. Intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mediate adhesion of monocytes to vascular endothelium and transmigration into the subendothelial space. CAMs are not constitutively expressed on the surface of normal endothelial cells. Their cellular expression is upregulated by a variety of cytokines in various conditions associated with endothelial activation including atherosclerosis [5].

**Table 1** Flow cytometric analysis of the influence of linoleic acid (10 µmol/l) and palmitic acid (10 µmol/l) on IL-1α (10 ng/ml) induced ICAM-1, VCAM-1 and E-selectin expression by endothelial cells HCAEC. The data represent mean values of four experiments

| | Fluorescence intensity | | |
|---|---|---|---|
| | ICAM-1 | VCAM-1 | E-selectin |
| Control | 408±56 | 189±64 | 270±40 |
| IL-1α | 693±61 | 341±77 | 410±54 |
| IL-1α and linoleic acid | 537±71* | 237±57* | 379±86 |
| IL-1α and palmitic acid | 655±121 | 365±28 | 391±85 |

* $P < 0.05$ compared to cells stimulated with IL-1α

Upregulation of CAMs expression is accompanied by the release of soluble forms of CAMs into the bloodstream. The levels of circulating CAMs have been shown to be increased under conditions in which tissue-expression of membrane-bond CAMs is also known to be increased. Elevated levels of circulating CAMs were found in patients with cardiovascular diseases and in subjects with several risk factors for atherosclerosis, including diabetes mellitus and insulin resistance [6–8]. In contrast, vegetarians have a diminished risk for the development of atherosclerotic diseases [15]. Our own preliminary results have shown the tendency of diminished concentrations of circulating CAMs in vegetarians.

Different lipoproteins and fatty acids may modulate cell responsiveness to cytokines. Since linoleic acid is implicated in several vasoprotective effects with respect to a healthy diet [1, 2], the influence of linoleic acid in comparison with palmitic acid regarding the CAM expression on normal human coronary artery endothelial cells was evaluated. The present study demonstrates that linoleic acid can inhibit the expression of cytokine-induced ICAM-1 and VCAM-1 on primary coronary artery endothelial cells. This is in accordance with effects of unsaturated fatty acids on CAM expression on the surface of veinous endothelial cells [9].

In addition to other monosaturated and polyunsaturated fatty acids, linoleic acid may contribute to the prevention of atherosclerotic diseases among other mechanisms through a modulation of CAM expression involved in the adherence of monocytes to vascular endothelium. Interestingly, the concentration of linoleic acid required to inhibit IL-1α-stimulated CAM expression on endothelial cells lies within the range of fatty acid concentration *in vivo* during administration of diet with high linoleic acid content.

Recently, De Catarina et al. [9] concluded, from studies with different fatty acids and numbers of double bonds, that the attenuation of CAM expression in cytokine-stimulated endothelial cells may be a function of the number of double bonds in the acyl chain. They observed that the incorporation of unsaturated fatty acids

in total endothelial cell lipids – at the expense of saturated fatty acids – inhibits the expression of CAMs.

The mechanism of fatty acid action in these and our experiments may include a reduced activation of the most important transcription factor controlling activation of endothelial cells, nuclear factor-kappa B, which upon activation translocates to the nucleus and activates gene transcription of CAM mRNA and ultimately *de novo* CAM synthesis [10, 12].

The results of the present study demonstrate that reduced CAM expression may be relevant to the antiatherogenic properties of linoleic acid. This is in contrast to the properties of palmitic acid. Linoleic acid as a component of a wholesome diet may contribute to the prevention of atherosclerosis through the modulation of expression of endothelial leukocyte adhesion molecules.

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