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TRANS FATTY ACIDS INDUCE APOPTOSIS IN HUMAN ENDOTHELIAL CELLS.

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The present study was designed to investigate the hypothesis that trans fatty acids can induce apoptosis of human umbilical vein endothelial cells (HUVEC). To test this hypothesis apoptosis was measured in HUVEC treated with 0.1, 1.0 or 5.0 mM trans elaidic acid (t-18:1) or linoelaidic acid (t,t-18:2) for 24 hours. For the detection of apoptosis, TdT-mediated dUTP nick end labelling assay (TUNEL), cell binding of annexin V and propidium iodide uptake were measured. Active Caspase-3 and cleaved PARP (poly-ADP-ribose polymerase) were also measured in the cell lysate. Moreover, cellular ability to produce ROS (reactive oxygen species) was measured by DCF fluorescence Both acids studied induce both early (annexin-positive cells) and late stages of apoptosis (cells stained by propidium iodide) in a dose-dependent manner. Also the appearance of TUNEL-positive cells was induced by both trans fatty acids tested, in a dose dependent manner. Both trans acids induce apoptosis through their effect on Caspase-3 activity and on intracellular ROS production. It is worth emphasising that linoelaidic acid proved to be a more potent inducer of apoptosis and ROS production in endothelial cells than elaidic acid.
The present studies suggest that trans fatty acids may play a role in damaging and death of vascular endothelial cells in atherosclerosis.

Key words: *atherosclerosis, endothelial cells, apoptosis, trans fatty acids*

INTRODUCTION

Endothelial cells of healthy vessels form an antithrombotic and anti-inflammatory barrier actively participating in maintenance of vascular haemostasis. It is well known that endothelial cell dysfunction plays an important role in the development and progression of atherosclerosis (1,2).

Apoptosis is a significant factor for normal development of the body and for maintenance of homeostasis, but also for the pathogenesis of many disorders, including atherosclerosis and its complications (3). The process of apoptosis absent in healthy vessels is a common phenomenon in advanced lesions and affects all types of cells forming the atherosclerotic plaque. Distribution of apoptosis in the atherosclerotic plaque is not uniform and most often is concentrated near macrophages, which suggests these cells participate in apoptosis initiation (4). Apoptotic endothelial cells have been detected on the lumenal surface of atherosclerotic coronary vessels but not in normal vessels, suggesting a link between endothelial apoptosis and the pathology of atherosclerosis (5).

The studies performed to date show that known pathogenic factors of atherosclerosis such as oxyLDL, oxygen free radicals, hyperglycaemia, arterial hypertension, or pro-inflammatory cytokines induce apoptosis of endothelial cells, while protective factors such as laminar flow, nitrogen oxide, growth factors or HDL inhibit the process of programmed cellular death (6).

The increased level of apoptosis of endothelial cells seems to be a particularly negative phenomenon. It may lead to exposure of the atherosclerotic lesion and collagen fibres, and to the loss of anticoagulant properties of the endothelium (3). Moreover, phosphatidylserine exposed during apoptosis on the surface of cells and apoptotic bodies exert potent pro-coagulant action. Apoptosis is suggested to play an important role in rupture of the atherosclerotic plaque and in thrombus formation, and thus in acute coronary events.

An increased interest in the effect of the diet containing trans fatty acids on the progression of cardiovascular disorders has been observed in the recent years. Epidemiological studies conducted to date show that increased TFA consumption of up to 2% of total energy was associated with an increased risk of coronary heart disease (7). The association between TFA intake and CHD risk was stronger than proven for the association between the intake of saturated fatty acids and CHD risk. In comparison with saturated fatty acids ingested at the same quantities, the above risk for TFA was 2.5 to 10 times higher. A strong positive association was also observed between 25-year death rates from coronary heart disease and average intake of the trans fatty acid, elaidic acid (8). Although the exact mechanism has not been fully elucidated, the available studies show that TFAs have a negative effect on lipid metabolism. Increased intake of TFA was proven to be associated with increased LDL cholesterol (9), reduced HDL cholesterol (10), and increased plasma levels of Lp(a) (11) and triglycerides (12). De Roos et al (10) suggest that the increased risk of CHD is associated with a negative effect of TFA on vascular endothelium. They proved that the replacement of dietary saturated fatty acids by trans fatty acids decreased serum HDL cholesterol and impaired flow-mediated vasodilatation. Recently, it has been shown that TFA intake is positively associated with systemic inflammation markers in women (13).

TFA contained in food are formed mainly in the process of industrial hardening of vegetable oils. Margarine and the shortenings have been the main

contributors to the intake of industrially-produced TFA. They contain 6.8% to 41% TFA (14). The major trans isomeric acid in hydrogenated fat is elaidic acid. Linoelaidic acid is present in partially hydrogenated vegetable oils.

Many cellular functions may change along with the change in the composition of fatty acids contained in cell membrane phospholipids (15). TFA is being incorporated into cell membrane phospholipids which impairs their function, especially the functions of ion channels. Only few studies assessing the effect of TFA on endothelial cells have been published so far.

The objective of our study is to verify whether the adverse effect of TFA on CHD risk is partially caused by their effect on apoptosis of endothelial cells.

MATERIALS AND METODS

Endothelial cell culture and experimental conditions

HUVEC were obtained from umbilical cords by collagenase digestion as described (16). In brief, veins of umbilical cords were perfused with PBS to remove blood cells, filled with 0.1% collagenase, and then left for 10 minutes at 37°C. The resulting cellular suspension was supplemented with FBS and centrifuged (400 x g) for 10 minutes. Afterwards, cells were transferred into 25-cm^2 flasks or 6-well tissue culture plates previously coated with Endothelial Cell Attachment Factor (Sigma Aldrich). HUVEC were cultured in EBM-2 medium with EGM-2 bullet kit containing 2% FBS, antibiotics and a mixture of growth factors as in the supplier's instruction, at 37°C under humidified 5% CO^2. The medium was replaced every 2 days until confluence (3-5 days). HUVEC purity was ascertained by the cobblestone morphology typical for quiescent endothelial cells and by staining for PECAM-1 (CD31). All experiments were performed using HUVEC at passage 3 or 4. After reaching the confluence medium EBM-2 was changed to Medium-1999 supplemented with 10% FBS and antibiotics and the cells were treated for 24 hours with 0.1, 1.0 and 5.0 mol/L elaidic acid or linoelaidic acid. Then the cells were harvested from the wells by trypsin digestion. Attached cells were pooled with any detached cells from the supernatant and pelleted by centrifugation.

Annexin V and propidium iodide staining

The effect of trans fatty acids exposure on HUVEC was determined by dual staining with Annexin V:FITC and propidium iodide, using the commercially available kit (Annexin V-FITC Apoptosis Detection Kit I; BD Biosciences Pharmingen). Annexin V:FITC and propidium iodide (PI) were added to the cellular suspension as in the manufacturer's instructions, and sample fluorescence of 10,000 cells was analysed by flow cytometry (Becton Dickinson).

Cells which were Annexin V:FITC positive and PI negative were identified as early apoptotic. Cells which were Annexin V:FITC positive and PI positive were identified as cells in late apoptosis or necrotic.

Identification of DNA fragmentation

The assay was performed by TdT-mediated dUTP Nick End Labelling (TUNEL) method using the APO-DIRECT™ kit (BD Pharmingen). Following the experiments, the HUVECs in suspension (1x 10^6/ml) were fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS), washed in

PBS, and suspended in 70% (v/v) ice-cold ethanol. The cells were stored in ethanol at - 20°C until use. The positive and negative controls and the samples were stained with FITC-dUTP by incubation in terminal deoxynucleotidyl transferase buffer as in the manufacturer's instructions, and sample fluorescence of 10,000 cells was analysed by flow cytometry (Becton Dickinson). Results are given as % of TUNEL-positive cells.

Measurement of active caspase-3 and cleaved PARP protein levels

Active caspase-3 and cleaved PARP (polymerase poli(ADP) rybose) protein levels were measured by using the commercially available kit BD™ Cytometric Bead Array (BD Biosciences Pharmingen).

The method principle is based on the formation of sandwich complexes similarly as in ELISA. The array of reagents contains a bead population with distinct fluorescence intensities coated with capture antibodies specific for active caspase-3 and cleaved PARP. After the cells (1 x 10^6) were treated as indicated, their cytosolic extract was prepared as in the manufacturer's instructions. The capture beads, PE-conjugated detection reagents, and cell lysate standard or the test samples are incubated together to form sandwich complexes. Following acquisition of sample data using flow cytometry, the sample results are generated in graphical and tabular format using BD™ CBA Analysis Software. Results are given as pg/ 1x10^6 cells.

Detection of intracellular reactive oxygen species (ROS) production

Intracellular ROS production was monitored by flow cytometry (Becton Dickinson) using method based on reactive oxygen species conversion of 2',7'-dichlorofluorescin (DCFH) to 2',7'-dichlorofluorescein (DCF) (17). The dye is applied to cells as 2',7'-dichlorofluorescein diacetate (DCFH DA,). Briefly, cells (3x10^5/ml) were treated with indicated concentration of elaidic acid or linoelaidic acid and than co-incubated with 50 μmol/L DCFH DA (Molecular Probes, Inc) for 1.5 hours. After incubation, the cells were re-suspended in ice-cold PBS, placed on ice in the dark, and analysed by flow cytometry. Results are given as mean fluorescence intensity.

RESULTS

Effect of trans fatty acids on early and late apoptosis stages

In order to check whether the acids studies induce apoptosis in endothelial cells, their effect on early apoptosis stages was assessed by measurement of the quantity of Annexin-positive cells, and their effect on late apoptosis stages was assessed by measurement of the quantity of cells stained by propidium iodide.

Both acids studied induce apoptosis of endothelial cells in a dose-dependent manner, and linoelaidic acid proved to be a much more potent inducer of both early and late stages of apoptosis (*Fig. 1* and *2*). Incubation of endothelial cells with 0.1 mmol/L, 1.0 mmol/L, and 5.0 mmol/L elaidic acid caused an approximately 2-fold increase (from 3.2 ± 0.8% to 8.0 ± 3.0%), an approximately 3-fold increase (from 3.2 ± 0.8% to 10.2 ± 2.8%), and an approximately 6-fold increase (from 3.2 ± 0.8% to 19.0 ± 5.2%), respectively, in % of tested cells in early stages of apoptosis (*Fig.1*). The quantity of cells at late stages of apoptosis increased similarly. 0.1, 1.0 and 5.0 mmol/L elaidic acid caused an approximately 4-fold increase (from 2.7 ±

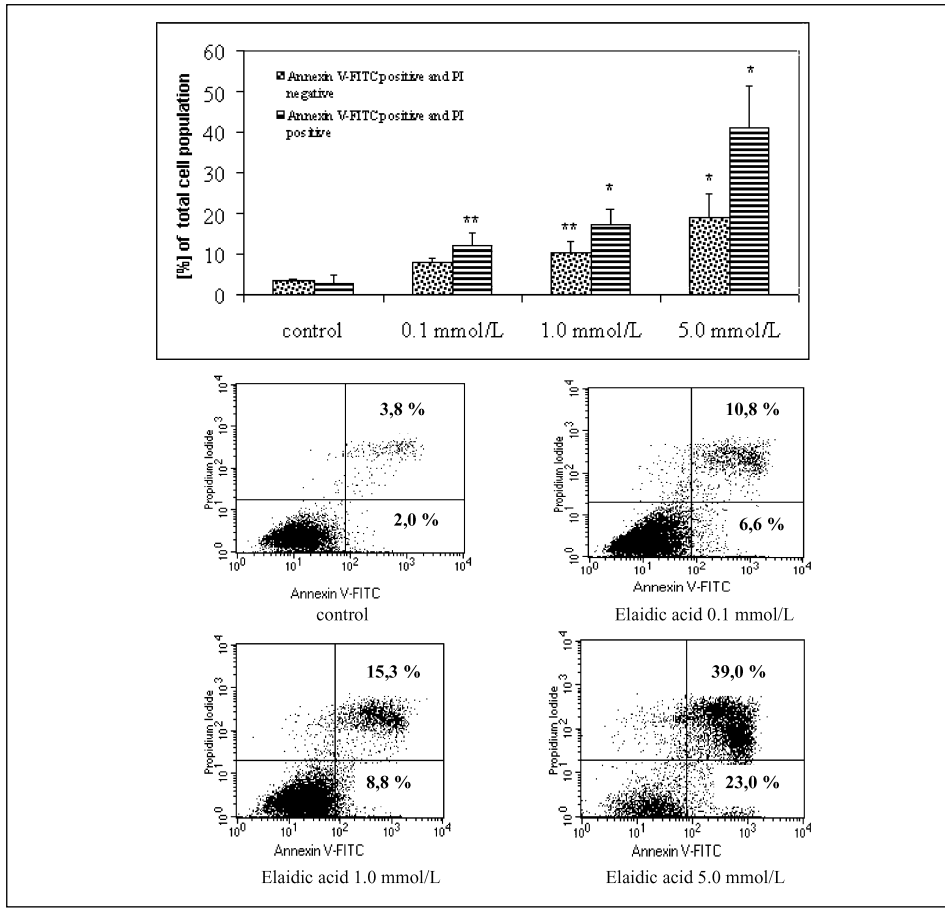

Fig.1 The effect of elaidic acid on either early or late stage of HUVEC apoptosis as detected by flow cytometry. HUVEC were incubated in Medium 199 supplemented with 10% FBS without (control) or with 0.1, 1.0 and 5.0 mmol/L elaidic acid. After 24 hours, the cells were harvested, stained with Annexin V-FITC and propidium iodide, and analysed by flow cytometry. Data are expressed as % of Annexin V-FITC- and PI-negative cells (early state of apoptosis) and as % of Anexin V-FITC- and PI-positive cells (late stage of apoptosis or necrosis). Diagrams of FITC-Annexin V/PI flow cytometry in a representative experiment are presented below the graphs. The lower right quadrants represent the cells in the early stage of apoptosis. The upper right quadrants contain the cells in the late stage of apoptosis or necrosis. ** $p < 0.01$, *$p < 0.001$ as compared with control.

0.9% to 11.8 ± 4.0%), an approximately 6-fold increase (from 2.7 ± 0.8% to 17.2 ± 4.7%), and an approximately 10-fold increase (from 2.7 ± 0.9% to 41.2 ± 4.7%), respectively, in % of cells stained by propidium iodide.

The results illustrating the effect of linoelaidic acid on early and late stages of apoptosis are presented in *Fig. 2*.

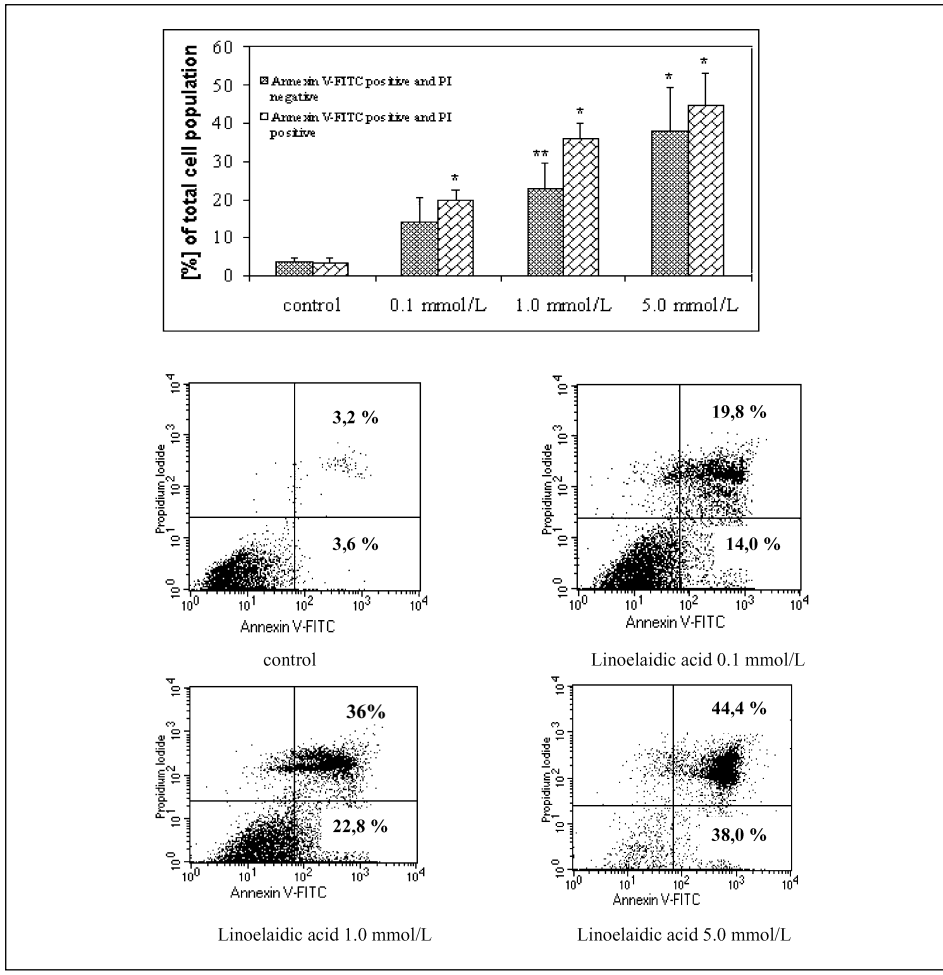

Fig.2 The effect of linoelaidic acid on either early or late stage of HUVEC apoptosis as detected by flow cytometry. HUVEC were incubated in Medium 199 supplemented with 10% FBS without (control) or with 0.1, 1.0 and 5.0 mmol/L linoelaidic acid. After 24 hours, the cells were harvested, stained with Annexin V-FITC and propidium iodide, and analysed by flow cytometry. Data are expressed as % of Annexin V-FITC- and PI-negative cells (early stage of apoptosis) and as % of Anexin V-FITC- and PI-positive cells (late stage of apoptosis or necrosis). Diagrams of FITC-Annexin V/PI flow cytometry in a representative experiment are presented below the graphs. The lower right quadrants represent the cells in the early stage of apoptosis. The upper right quadrants contain the cells in the late stage of apoptosis or necrosis. ** $p < 0.01$, *$p < 0.001$ as compared with control.

Incubation of endothelial cells with 0.1 mmol/L, 1.0 mmol/L, and 5.0 mmol/L linoelaidic acid caused an approximately 4-fold increase (from 3.6 ± 0.9% to 14.0 ± 6.5%), an approximately 6-fold increase (from 3.6 ± 0.9% to 22.8 ± 6.5%), and an approximately 11-fold increase (from 3.6 ± 0.9% to 38.0 ± 11.5%),

respectively, in the quantity of cells at early stages of apoptosis. 0.1 mmol/L, 1.0 mmol/L, and 5.0 mmol/L linoelaidic acid caused an approximately 6-fold increase (from 3.2 ± 1.3% to 19.8 ± 2.6%), an approximately 11-fold increase (from 3.2 ± 1.3% to 36.0 ± 4.1%), and an approximately 14-fold increase (from 3.2 ± 1.3 to 44.6 ± 8.6%), respectively, in % of cells stained by propidium iodide.

The lower parts of *Fig. 1* and *2* contain the histograms of typical experiments conducted for control cells and for cells subjected to the action of the tested trans acids at various doses. In TFA-treated cultures, the populations of apoptotic cells were markedly distinguishable from the overall cell population.

Effect of trans fatty acids on DNA fragmentation

To confirm that TFA can induce endothelial cell apoptosis, DNA fragmentation measured by TUNEL method was also studied after HUVEC exposure to trans fatty acids. The appearance of TUNEL-positive cells was induced by both trans fatty acids tested, in a dose dependent manner (*Fig. 3* and *4*). In contrast, very few TUNEL-positive cells were observed in the population of control cells. Similarly as it had been observed in the case of the effect of the tested acids on early and late stages of apoptosis, elaidic acid proved to be a weaker inducer of DNA fragmentation in comparison with linoelaidic acid. Treatment of endothelial cells with elaidic acid changed the percentage of TUNEL-positive cells from 1.3 ± 0.3% (control cells) to 8.6 ± 1.7% (0.1 mmol/L), to 13.1 ± 2.9% (1.0 mmol/L), and to 20.5 ± 4.7% (5.0 mmo/L). And in the case of endothelial cells incubated with linoelaidic acid, an increase in quantity of TUNEL-positive cells from 1.3 ± 0.3% to 17.3 ± 3.2% (0.1 mmo/L), from 1.3 ± 0.3% to 20.6 ± 3.3% (1.0 mmol/L), and from 1.3 ± 0.3% to 70.6 ± 8.3% (5.0 mmol/L) was observed.

Effect of trans fatty acids on active caspase-3 and cleaved PARP protein levels

To identify the regulatory pathways of endothelial cell apoptosis induced by trans fatty acids, the activity of caspase-3 was measured in HUVEC treated with these fatty acids for 24 hours. As shown in *Fig. 5*, the exposure to elaidic acid resulted in a dose-dependent increase in caspase-3 activity. 0.1, 1.0 and 5.0 mmol/L elaidic acid caused an approximately 4-fold increase (from 35.6 ± 6.0 pg/1x10^6 to 150.4 ± 25.6 pg/1x10^6), an approximately 6-fold increase (from 35.6 ± 6.0 pg/1x10^6 to 204.0 ± 29.6 pg/ 1x10^6), and an approximately 8-fold increase (from 35.6 ± 6.0 pg/1x10^6 to 292.4 ± 24.6 pg/1x10^6), respectively, in the intracellular level of active caspase. In contrast, linoelaidic acid had a much more potent effect on the quantity of active caspase-3 in the cells tested, but only at 0.1 mM and 1.0 mM doses. An approximately 6-fold increase in quantity of active caspase-3 in the case of 0.1 mM concentration was found from 35.6 ± 6.0 pg/1x10^6 to 219.2 ± 30.6 pg/ 1x10^6 and an approximately 27-fold increase in the case of 1.0 mM concentration from 35.6 ± 6.0 pg/1x10^6 to 975.6 ± 29.7 pg/ 1x10^6 cells (n=5' $p < 0.001$). The level of active caspase-3 in cells incubated with 5.0

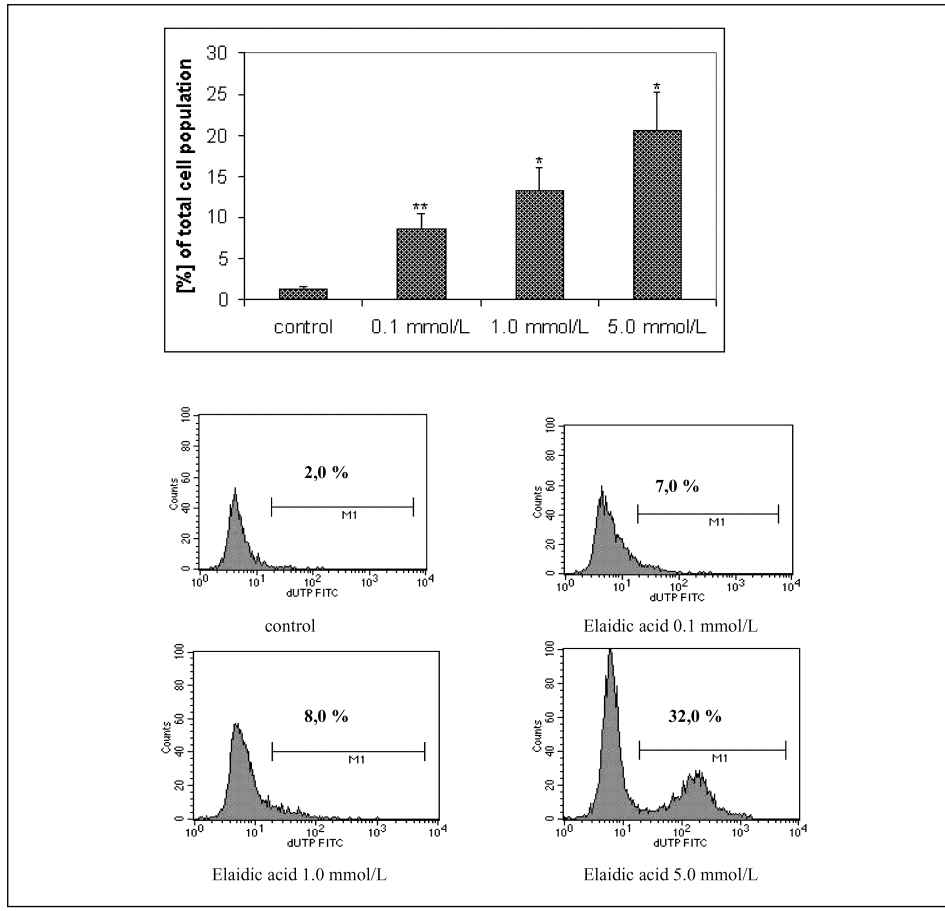

Fig. 3 Elaidic acid induces DNA fragmentation in endothelial cells. HUVEC were incubated in Medium 199 supplemented with 10% FBS without (control) or with 0.1, 1.0 and 5.0 mmol/L elaidic acid for 24 hours, and assayed by the TUNEL method. The results are expressed as % of TUNEL-positive cells and are given as the mean ± SD for five experiments. Typical flow cytometry analysis histograms in a representative experiment are presented below the graphs. ** $p < 0.01$, *$p < 0.001$ as compared with control.

mM linoelaidic acid was 293 ± 24.4 pg/1x10^6 and was lower in the case of cells incubated with this acid at concentration of 1.0 mmol/L.

The above results are consistent with the results assessing the effect of elaidic and linoelaidic acids on the content of the degraded form of PARP which is one of substrates for active caspase-3, presented in *Fig. 6.* Incubation of endothelial cells with 0.1 mM, 1.0 mM, and 5.0 mM elaidic acid was associated with an approximately 5-fold increase from 29.8 ± 5.8 pg/1x10^6 to 150 ± 16.6 pg/1x10^6; an approximately 6-fold increase from 29.8 ± 5.8 pg/1x10^6 to 177.4 ± 9.6

pg/1x10^6); an approximately 10-fold increase from 29.8 ± 5.8 pg/1x10^6 to 286.6 ± 46.1 pg/1x10^6, respectively, in the quantity of degraded PARP.

On the other hand, incubation of endothelial cells with 0.1 mM and 1.0 mM linoelaidic acid was associated with an approximately 6-fold increase from 29.8 ± 5.8 pg/1x10^6 to 166 ± 25.1 pg/1x10^6 and an approximately 33-fold increase from 29.8 ± 5.8 pg/1x10^6 to 998.8 ± 40.3 pg/1x10^6, respectively, in the quantity of degraded PARP. Similarly as in the case of caspase-3, the quantity of degraded

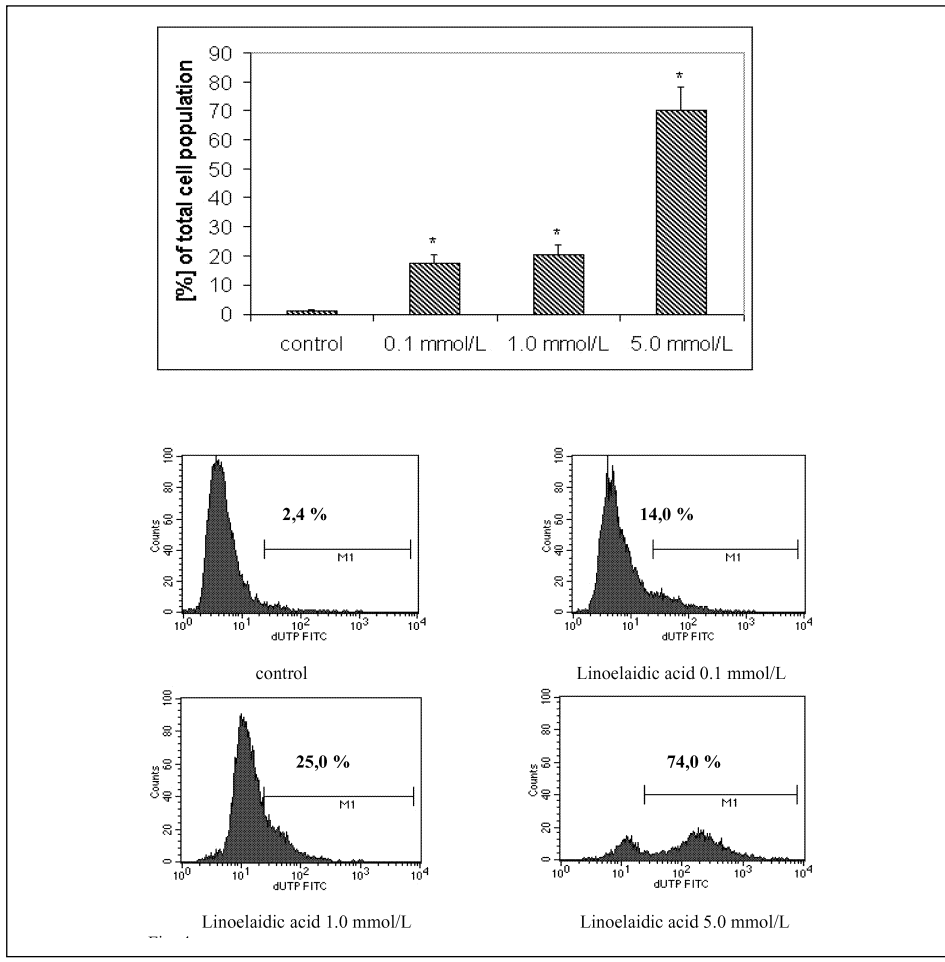

Fig. 4 Linoelaidic acid induces DNA fragmentation in endothelial cells. HUVEC were incubated in Medium 199 supplemented with 10% FBS without (control) or with 0.1, 1.0 and 5.0 mmol/L linoelaidic acid for 24 hours, and assayed by the TUNEL method. The results are expressed as % of TUNEL-positive cells and are given as the mean ± SD for five experiments. Typical flow cytometry analysis histograms in a representative experiment are presented below the graphs. *p <0.001 as compared with control.

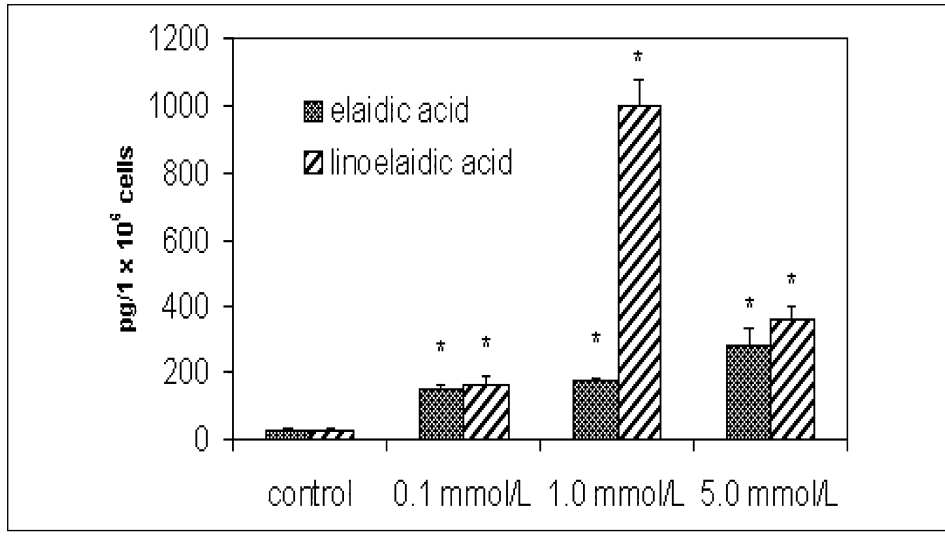

Fig.5. The effect of elaidic and linoelaidic acids on active caspase-3 protein levels. HUVEC were incubated in Medium 199 supplemented with 10% FBS without (control) or with 0.1, 1.0 and 5.0 mmol/L elaidic acid or linoelaidic acid. After 24 hours, the cells were harvested and lysed. Cytosolic extract was used for determination of active caspase-3 protein levels as described in Materials. The results are expressed as pg/ 1 x 10^6 and are given as the mean ± SD for five experiments *p <0.001 as compared with control.

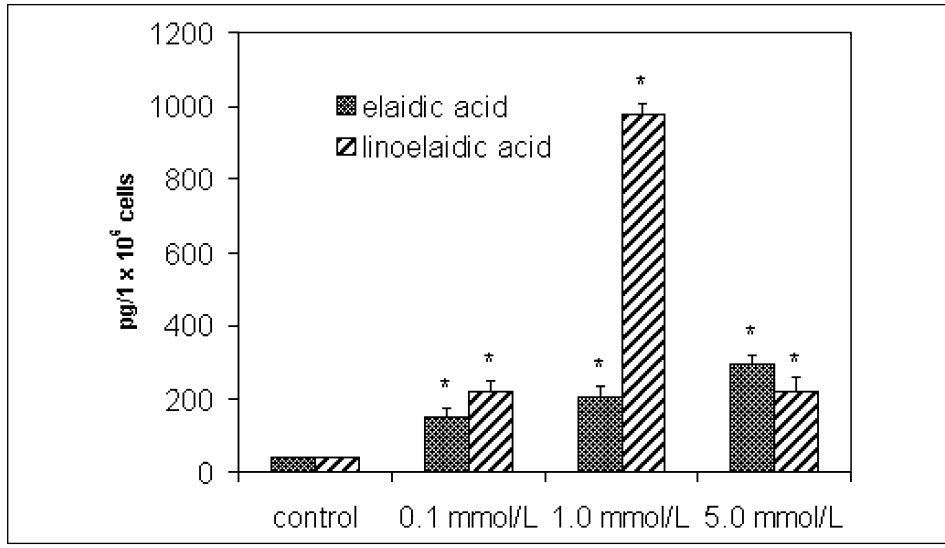

Fig.6. The effect of elaidic and linoelaidic acids on cleaved PARP protein levels. HUVEC were incubated in Medium 199 supplemented with 10% FBS without (control) or with 0.1, 1.0 and 5.0 mmol/L eliadic acid or linoelaidic acid. After 24 hours, the cells were harvested and lysed. Cytosolic extract was used to determining of cleaved PARP protein levels as described in Materials. The results are expressed as pg/ 1 x 10^6 and are given as the mean ± SD for five experiments *p <0.001 as compared with control.

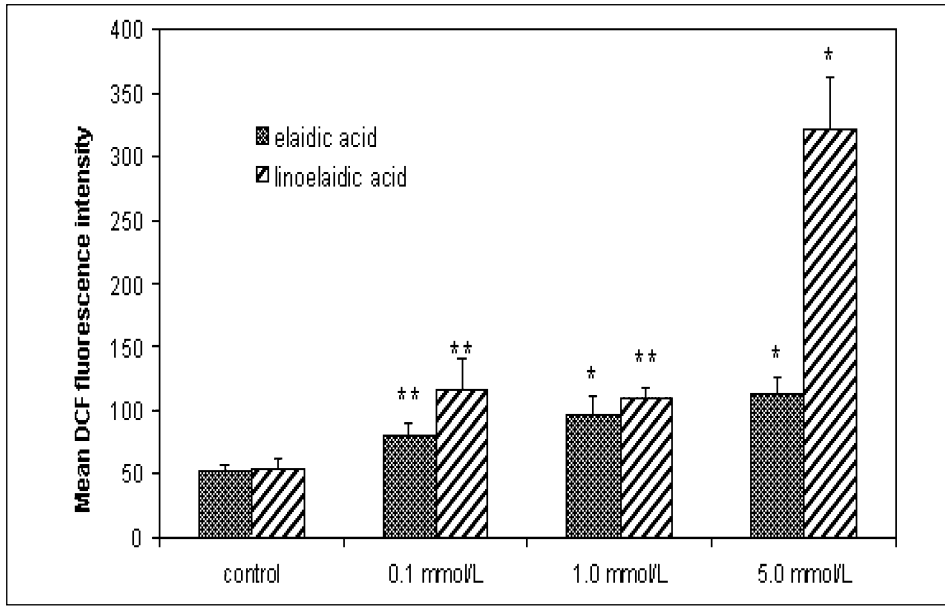

Fig.7 Elaidic and linoelaidic acids induced intracellular ROS production in HUVEC. HUVEC were exposed to elaidic acid or linoelaidic acid (0.1, 1.0 and 5.0 mm0l/L) and DCFH DA (50 µmmol/L) for 1.5 hours. Control cells were exposed only to DCFH DA. Intracellular ROS production was determined by DCF fluorescence as described in Methods. Results are expressed as the Mean Fluorescence Intensity and are given as the mean ± SD for six experiments. **p < 0.01, *p < 0.001 as compared with control.

PARP in cells incubated 24 hours with 5.0 mM linoelaidic acid was lower than in the cells incubated with this acid at concentration of 1.0 mM and was 356.8 ± 40.3 pg/1 x10^6. It is suggested that in the case of 5.0 mM linoelaidic acid, caspase-3 is activated much earlier.

Effect of trans fatty acids on intracellular ROS production

Because it is generally accepted that oxidative stress may invoke apoptotic cell death, we decide to check also the influence of elaidic and linoelaidic acids on intracelullar ROS production. The cellular ability to produce ROS was measured by DCF fluorescence. This reaction is initiated primarily by peroxides, mainly hydrogen peroxide (17). Similarly as in the case of apoptosis treatment of HUVEC, elaidic acid was found to increase DCF fluorescence in a dose-dependent manner as compared to control. Intracellular ROS production increased from 51.8 ± 4.4 (control cells) to 80.0 ± 10.3 (0.1 mmol/L), to 97.8 ± 14.7 (1.0 mmol/L) and to 112.7 ± 13.7 (5.0 mmol/L).

Similarly, linoelaidic acid in a dose-dependent manner stimulated RFT production, and its effect at 5.0 mM was much stronger than the effect of elaidic

acid. In the case of endothelial cells incubated with linoelaidic acid, DCF fluorescence increased from 51.8 ± 4.4 in the control test to 115.5 ± 25 (0.1 mmol/L), to 109.7 ± 14.7 (1.0 mmol/L), and to 320.8 ± 42.3 (5.0 mmo/L).

DISCUSSION

The recent studies suggest that the process of apoptosis plays an important role in the pathogenesis of atherosclerosis and significantly contributes to rupture of the atherosclerotic plaque (3). Apoptosis of endothelial cells seems to have an especially negative effect on the progression of atherosclerosis, as it initiates erosion of the plaque and contributes to the hypercoagulation state.

In our own studies we have shown for the first time that trans fatty acids may induce apoptosis of endothelial cells. The studies conducted have proven that both elaidic and linoelaidic acids stimulate in a dose-dependent manner early and late stages of apoptosis. Out of two acids studied, linoelaidic acid proved to be a more potent inducer of apoptosis.

In most cases, an approximately 2-fold increase in the percentage of cells in late stages of apoptosis - i.e. cells stained by both Annexin as well as by propidium iodide - in comparison with the cells at early stages of apoptosis. On one hand, this may suggest a strongly toxic action of the acids studied and thus rapid initiation of the effective pathways of apoptosis. On the other hand, it may result from desquamation of phosphatidylserine from the cellular surface, and thus a weaker reaction with Annexin. Recently, studies have been published demonstrating that induction of apoptosis of endothelial cells with camptothecin is associated with the release of Annexin V-binding micro-particles from the cellular surface (18).

Apart from rearrangement of the cellular membrane, one of the best known characteristics of apoptotic cells is DNA fragmentation measured most often by the TUNEL method (19). In our studies we have proven that incubation of endothelial cells with elaidic and linoelaidic acids is associated with an increase in the percentage of TUNEL-positive cells.

Lima et al (20) conducted experiments aimed at demonstrating the cytotoxic effect of fatty acids on lymphocytes B and T. Cytotoxicity of the acids studied depended on their chain length and from the number of double bonds. 24-hour incubation of Jurkat cells with 0.4 mM elaidic acid was associated with the vitality loss by approximately 33% of cells, and in the case of Raji cells, 24-hour incubation with this acid at a concentration of 0.5 mM caused vitality loss by 12% of cells. The above results, despite quantitative differences, are consistent with the results obtained in our own studies in which 24-hour incubation of endothelial cells with 0.1 mM elaidic acid was associated with vitality loss by 15% of cells. These quantitative differences are undoubtedly caused by the differences in concentrations and also by the different response of individual cellular lines to

apoptosis-inducing factors. Endothelial cells, especially in the confluent state, i.e. in the resting state - when not proliferating - are weakly sensitive to apoptosis. Camptothecin, a topoisomerase inhibitor, stimulates at a concentration of 5.0 μM apoptosis in Jurkat cells within only 4 - 6 hours. On the other hand, a period of 24 hours is necessary for inducing apoptosis of endothelial cells with the use of camptothecin at the same concentration (18).

In order to partially explain the effect of the acids tested on endothelial cells, a decision was made to assess their effect on activity of caspase-3, the main executor of apoptosis. Incubation of endothelial cells with elaidic acid and linoelaidic acid led to activation of caspase-3 and thus to an increase in the quantity of cleaved PARP which is one of substrates for this enzyme.

It is worth emphasising that many known pathogenic factors of atherosclerosis exert a pro-apoptotic action on endothelial cells through their effect on the activity of caspase-3, the main effector of apoptosis. Umera et al (21) showed that oxy LDL induced apoptosis in endothelial cells through their effect on activity of caspase-3 and production of reactive oxygen species. Similarly, hyperglyceridemia induces apoptosis in endothelial cells through an effect on ROS production and caspase-3 activation (22). As it is known, hyperhomocysteinemia is a well-established risk factor of atherosclerosis. As it arises from the recently published studies, also homocysteine induces apoptosis in endothelial cells through its effect on caspase-3 activity (23).

It is a generally accepted view that oxidative stress induces programmed cellular death, and an inhibition or delay of apoptosis may be obtained with the use of antioxidants (24). Many mechanisms are proposed through which ROS may induce apoptosis. It is known that ROS initiate double breakages of DNA which may be a factor which directly activates apoptosis. DNA damages activate poly(ADP-ribose) polymerase, which leads to a reduction in the intracellular pool of NAD/NADH and ATP concentration. Energy deprivation of a cell may be a direct cause of its death. ROS cause also oxidation of proteins which may be a direct cause of apoptosis (24). Also ROS and DNA damage is identified as a factor inducing the mitochondrial pathway of apoptosis.

In our own studies we have shown that both elaidic acid as well as and linoelaidic acid increase in a dose-dependent manner ROS production in endothelial cells which suggests this may be one of mechanisms inducing apoptosis in these cells.

An important role in pathogenesis of apoptosis is played by the intracellular concentration of Ca^{2+} ions (25). Increased levels of this ion may lead to activation of Ca^{2+} -dependent kinases or phosphatases, with subsequent activation of genes related to the apoptotic process. Increased levels of Ca^{2+} in cytosol result also in their increased capture by mitochondria, and this in turn result in increased release of cytochrome C and thus in activation of the endogenous apoptotic pathway (26). Recently, studies have been published which show that the presence of elaidic and linoelaidic acids in the incubation medium is associated

with incorporation of these acids into endothelial cells with a concomitant increase in incorporation of Ca^{2+} ions (27) .

In the USA, Canada and in the Western European countries, the consumption of products containing trans fatty acids has been decreasing since 1996. Nevertheless, trans fatty acids are constantly present in many popular products such as doughnuts, crisps, instant soups, popcorn, etc., in quantities exceeding 10 g of TFAs per serving. Therefore, it may be hypothesised that their presence in the postprandial lipeamia, i.e. in chylomicrons, contributes to TFA accumulation not only in adipocytes, but also in the vascular wall, including atherosclerotic plaques (28).

The presented study results suggest that trans fatty acids contained in certain food products may lead to death of endothelial cells *inter alia* due to their effect on the activity of the main effector enzyme, i.e. caspase-3, and on the intracellular production of reactive forms of oxygen. Thus they may play an important role in progression of atherosclerosis.

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