7 Summary

Several *in vivo* studies suggest that conjugated linoleic acid (CLA) isomers occurring naturally in milk and meat of ruminants influence atherosclerotic processes. Atherosclerosis constitutes the most important contributor to cardiovascular diseases representing the leading cause of death in developed countries. Nutrition plays a pivotal role in the development of atherosclerosis-risk factors such as overweight, lipometabolic disorders, hypertension and diabetes mellitus.

The aim of this study was to investigate the effects of cis-9, trans-11 CLA and trans-10, cis-12 CLA on atherosclerosis-relevant parameters, vasoactive substances and pro-inflammatory parameters, in human aortic endothelial cells.

To investigate the effects of both CLA isomers on the release of vasoactive substances nitric oxide (NO), endothelin-1 (ET-1) and eicosanoids prostacyclin (PGI₂), thromboxane (TX) A₂, prostaglandine (PG) E₂ and F₂α, human aortic endothelial cells (HAoEC) were incubated with 5 or 50 µmol/L of cis-9, trans-11 CLA and trans-10, cis-12 CLA for 24 h at 37°C. After the incubation period the fatty acid composition in total lipids and the phospholipid fractions phosphatidylethanolamine and phosphatidylcholine, formation of conjugated metabolites of CLA in total lipids, parameters of the eicosanoid synthesis and NO synthesis as well as the release of ET-1 were determined. Incubation of HAoEC with the CLA isomers resulted in a significant incorporation of either CLA isomer and a significant isomer-specific formation of conjugated metabolites of CLA (CD) such as CD16:2 and CD20:2 in a concentration dependent manner when compared with untreated endothelial cells. Endothelial cells incubated with 50 µmol/L with either cis-9, trans-11 CLA or trans-10, cis-12 CLA released less eicosanoids, NO and ET-1 than control cells. The ratio between the amounts of 6-keto-PGF₁α, and that of TXB₂ (as stable derivates of PGI₂ and TXA₂, respectively) released did not differ between control cells and cells treated with CLA isomers. Furthermore, cells treated with 50 µmol/L of either cis-9, trans-11 CLA or trans-10, cis-12 CLA had
lower amounts of arachidonic acid (AA) in phospholipid fractions, a reduced mRNA concentration and activity of secretory phospholipase A\textsubscript{2} compared with control cells. The ratio of the amounts of AA and those of linoleic acid (LA) as an index of $\Delta5$- and $\Delta6$-desaturation were lower in phospholipid fractions of endothelial cells treated with CLA isomers than those of untreated cells. Derived from investigations, CLA isomers have neither a positive nor a negative effect on the ratio between the relaxation and constriction factors, parameters of the vascular homeostasis, in endothelial cells.

To investigate the effect of both CLA isomers on inflammatory parameters of the early phase of atherosclerosis, endothelial cells were incubated in medium without (control) or with 5 or 50 $\mu$mol/L of either cis-9, trans-11, trans-10, cis-12 CLA or LA, as a reference substance, for 20 h at 37°C followed by a 4 h treatment with 2 ng/mL TNF\textalpha to cause an inflammatory situation. After the incubation periods, the expression of adhesion molecules intracellular adhesion molecule 1 (ICAM-1), vascular cellular adhesion molecule 1 (VCAM-1) and E-selectin, the release of chemokines monocyte chemoattractant or chemotactic protein-1 (MCP-1) and interleukin (IL)-8 and the monocyte adhesion to endothelial cells as well as the DNA-binding activity of the transcription factors nuclear factor-$\kappa$B (NF-$\kappa$B) and peroxisome proliferator-activated receptor (PPAR) $\gamma$ were determined. Treatment of HAoEC with 2 ng/mL TNF\textalpha increased release of the chemokines, expression of adhesion molecules and monocyte adhesion. However, treatment of HAoEC with either CLA isomer or LA did not modulate the cytokine-induced expression of ICAM-1, VCAM-1 and E-selectin, MCP-1 and IL-8 release and U937 cell adhesion. In addition, both CLA isomers and LA slightly increased PPAR$\gamma$ DNA-binding activity, but almost did not alter DNA-binding activity of NF-$\kappa$B. This suggests that CLA isomers had no effect on parameters of the early phase of atherosclerosis in HAoEC. This raises the possibility that the anti-atherogenic effects of CLA described in the literature might be explained by influence on processes of a later stage of atherogenesis or factors other than those addressed in this study.

In conclusion, both CLA isomers cis-9, trans-11 CLA and trans-10, cis-12 CLA had no effects on the investigated atherosclerosis-relevant parameters in human arterial endothelial cells.