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Review Article

Influence of conjugated linoleic acids on functional properties of vascular cells

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Conjugated linoleic acids (CLA) are biologically highly active lipid compounds that inhibit the development of atherosclerotic plaques in experimental animals. The underlying mechanisms of action, however, are only poorly understood. Since cell-culture experiments are appropriate to provide a detailed view into the mechanisms of action of a compound, the present review summarises results from *in vitro* studies dealing with the effects of CLA isomers and CLA mixtures on functional properties of cells of the vascular wall, such as endothelial cells, smooth muscle cells and monocyte-derived macrophages, which are amongst the major cells contributing to atherosclerotic lesion development. Based on these studies, it can be concluded that CLA exert several beneficial actions in cells of the vascular wall through the activation of nuclear PPAR. These actions of CLA, which may, at least partially, explain the inhibition of atherogenesis by dietary CLA, include modulation of vasoactive mediator release from endothelial cells, inhibition of inflammatory and fibrotic processes in activated smooth muscle cells, abrogation of inflammatory responses in activated macrophages, and reduction of cholesterol accumulation in macrophage-derived foam cells.

Conjugated linoleic acid: Atherosclerosis: Endothelium: Smooth muscle cells: Macrophages

Conjugated linoleic acids (CLA) are a group of positional and geometrical isomers of linoleic acid (18 : 2*n*-6) characterised by the presence of conjugated double bonds. A wide spectrum of different CLA isomers, which vary in position (most frequently 7 and 9, 8 and 10, 9 and 11, 10 and 12, 11 and 13) and geometry (*trans*, *trans*; *trans*, *cis*; *cis*, *trans*; and *cis*, *cis*), has been shown to occur naturally in food[1]. The most important sources of CLA in the human diet are ruminant-derived products (milk, dairy products, meat)[2–4], because CLA isomers are produced in the rumen during microbial biohydrogenation of dietary linoleic acid and in tissues through Δ9-desaturation of the rumen-derived *trans*-vaccenic acid (*trans*-11-18 : 1)[5–7]. The predominant CLA isomer in ruminant-derived products is *cis*-9, *trans*-11-CLA (also known as rumenic acid), contributing to more than 90 % of total CLA[2], and it is now accepted that endogenous synthesis by Δ9-desaturation contributes most to this CLA isomer in ruminant-derived products[6,7]. Since Δ9-desaturation of fatty acids also occurs in tissues of single-stomached species, non-ruminant-derived meat also contains CLA, but at a much lower content[8]. Endogenous formation of CLA by Δ9-desaturation also explains the finding that the concentration of CLA, in particular *cis*-9, *trans*-11-CLA, in cells and tissues of humans significantly increases in response to a diet rich in *trans*-11-18 : 1[9,10]. In addition to natural foodstuffs, dietary CLA supplements, which are available over the counter in supermarkets and drug stores as well as via the Internet, can also contribute to CLA intake in humans. In contrast to natural foodstuffs, dietary supplements have a different CLA isomeric profile. The main difference is the high percentage of *trans*-10, *cis*-12-CLA (up to 50 % of total CLA) in supplements[2], whereas this CLA isomer is only a minor component in dairy products or meat. According to recent studies, average daily intakes of CLA in Europe and the USA are estimated to be in the range of 100–400 mg[8,11–15]. In comparison, CLA intake from dietary CLA supplements marketed for weight-loss purposes is markedly higher (2–4 g/d), provided that the manufacturers' recommendations are followed.

CLA have attracted great scientific interest due to several beneficial properties[16–22]. Amongst these, the anti-atherogenic properties of CLA are of particular interest regarding the high prevalence of atherosclerosis, which is the principal cause of CHD and stroke and is responsible for more than 40 % of all deaths in middle Europe and the USA[23,24], and, thus, the need for developing strategies to

Abbreviations: AA, arachidonic acid; ABC, ATP-binding cassette; AP, activator protein; CLA, conjugated linoleic acid; COX, cyclo-oxygenase; ECM, extracellular matrix; ET-1, endothelin-1; ICAM, intercellular adhesion molecule; MMP, matrix-metalloproteinase; PAF, platelet activating factor; PLA_2, phospholipase A_2; VCAM, vascular cell adhesion molecule.

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prevent or treat atherosclerosis. Although there is convincing evidence that CLA (isomers or mixtures) exhibit anti-atherogenic effects in animal models[25,26], the preventive and therapeutic potential of CLA on atherosclerosis development in humans remains to be determined[26]. Most of the human studies reported no effect of supplementation with CLA (isomers or mixtures) on surrogate markers of atherosclerosis (inflammatory parameters, plasma lipid profile)[27–30], and some human studies even reported detrimental effects on risk factors associated with atherosclerosis[31–34]. It has been suggested that differences in purity, content of CLA isomers, and co-existence of other fatty acids in CLA supplements and/or differences in the CLA dose are responsible for the inconsistent data on the effects of CLA on atherosclerosis development in humans[26]. However, differences in the study design (limitation in diet and physical activity or not) and in the study collectives (healthy, obese or diabetic, male or female, young or old) might also contribute to these inconsistencies.

Besides these unresolved questions regarding the inconsistent data from human studies, there is also a lack of knowledge with respect to the mechanisms underlying the inhibitory effect of CLA on atherogenesis. However, recent studies demonstrated that CLA are able to bind to and activate PPAR[35–37]. The PPAR, from which the three isotypes α, β/δ and γ have been identified, are ligand-activated transcription factors that act as important regulators of lipoprotein metabolism, cholesterol and glucose homeostasis, and inflammation[38–41], all of which are important factors in atherosclerosis development. Transcriptional regulation of genes by PPAR is mediated by the binding of activated PPAR–retinoid X receptor heterodimers to specific DNA sequences, called peroxisome proliferator response elements present in and around the promoter region of target genes[42–44], thereby stimulating the expression of those genes[45,46] (Fig. 1). PPAR are also capable of negatively modulating gene expression by inhibiting DNA binding of several other transcription factors, such as NF-κB[40,47–50]. NF-κB plays a central role in inflammatory responses by the regulation of the transcription of genes involved in inflammation[51,52]. Inhibition of NF-κB by PPAR is of great importance for the modulation of atherosclerosis development, because it is well established that activation of NF-κB in vascular cells significantly contributes to the development of vascular disorders such as atherosclerosis or hypertension[53,54]. Hence, it is not surprising that PPAR ligands exert direct vascular-protective effects in cells of the vascular wall, which express all PPAR isotypes, by modulating the expression of genes implicated in the atherosclerotic process through the activation of PPAR[41,55]. Since CLA are also ligands of PPAR, it is plausible to speculate that the anti-atherogenic effects of CLA are, at least partially, mediated by the modulation of pro-atherogenic gene expression by PPAR in vascular cells.

Influence of conjugated linoleic acids on the function of cells of the vascular wall

The present review summarises the current knowledge about the effects of CLA on functional properties of cultivated cells of the vascular wall, such as endothelial cells, smooth

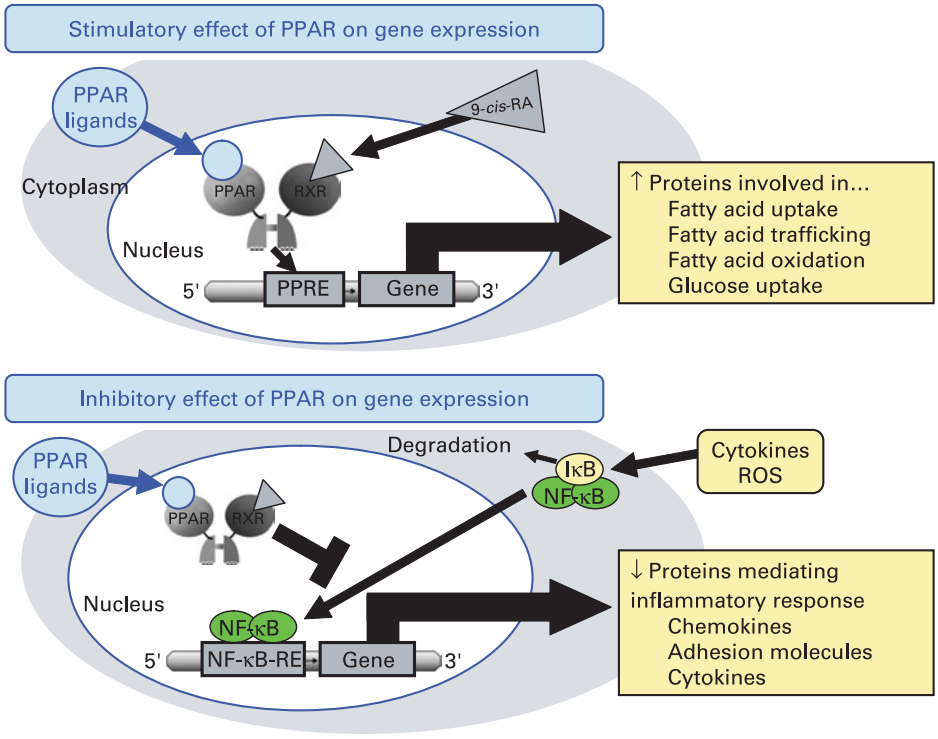

Fig. 1. Schematic presentation of the mechanisms of action of PPAR on gene transcription. PPAR stimulate gene transcription by binding of activated PPAR/retinoid X receptor (RXR) heterodimers to specific DNA sequences, called peroxisome proliferator response elements (PPRE) present in and around the promoter region of target genes, thereby stimulating the expression of those genes. PPAR also negatively modulate gene transcription by inhibiting DNA binding of several other transcription factors, such as NF-κB. 9-*cis*-RA, 9-*cis*-retinoic acid; IκB, inhibitor of κB; ROS, reactive oxygen species; RE, response element.

muscle cells and monocyte-derived macrophages, which are amongst the major cells contributing to atherosclerotic lesion development[56–58] (Fig. 2), taking PPAR-regulated processes into special consideration.

Influence of conjugated linoleic acids on endothelial cell function

Endothelial cells cover the lumen of the vessels, thereby forming a selectively permeable barrier, called endothelium, between the blood and the vascular tissue. Together with the sub-endothelial space consisting of connective tissue and a network of elastic fibres, the endothelium makes up the intima, the innermost layer of the vessel wall, which extends to the internal elastic lamina. The internal elastic lamina separates the intima from the media, where the vascular smooth muscle cells are located[59]. The vascular endothelium not only lines the vessels, but also plays a critical role in vessel wall regulation. Endothelial cells synthesise and release biologically active substances that control all aspects of the integrity and metabolism of the vascular wall, such as vascular structure and permeability, vascular tone and blood pressure, coagulation and fibrinolysis, and inflammatory response[60,61]. Accordingly, injury to the endothelium or disturbances of normal endothelial cell function, called endothelial dysfunction, have serious implications and lead to the development of atherosclerosis[62]. Hence, endothelial dysfunction is considered one of the critical events in the development of atherosclerosis.

Endothelial dysfunction is accompanied by an activation of endothelial cells leading to increased adhesion of circulating leucocytes (monocytes, lymphocytes) to the activated endothelium, and, subsequently, transendothelial migration of leucocytes into the sub-endothelial space[63]. Recruitment of circulating leucocytes to the activated endothelium is mediated by inflammatory chemokines such as IL-8 and monocyte chemoattractant protein-1, which are secreted locally in large amounts from activated endothelial cells. Leucocyte adhesion is mediated by inducible cell adhesion molecules such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and endothelial selectin, expressed at high levels on the surface of activated endothelial cells. Subsequently, infiltrated monocyte-derived macrophages with high phagocytic activity take up lipid-rich lipoprotein particles, which enter the sub-endothelial space due to the impaired barrier function of the activated endothelium. The resulting accumulation of lipids in the vessel wall leads to the first visible atherosclerotic lesions, called fatty streaks. Neovascularisation of atherosclerotic plaques also contributes to leucocyte infiltration of the vascular wall[64]. In fact, plaque neovasculature, which is found in most human atherosclerotic plaques[65], and consists of small vessels arising primarily from the adventitial vasa vasorum[66], represents a significant route for infiltration of leucocytes into advanced atherosclerotic plaques. This is based on the observation that the expression of adhesion molecules on neovascular endothelium is twice as high as on the endothelium of the arterial lumen[64].

Conjugated linoleic acid effects on leucocyte–endothelial cell interactions

Pharmacological ligands of PPAR were shown to reduce the cytokine-stimulated surface expression of adhesion molecules, leucocyte adhesion and chemokine release in endothelial cells[55,67–69]. These effects are largely explained by inhibitory effects of PPAR ligands on DNA binding of transcription factors such as NF-κB, activator protein (AP)-1 and signal transducers and activators of transcription (STAT)-3[40,47–50]. NF-κB is considered to be one of the

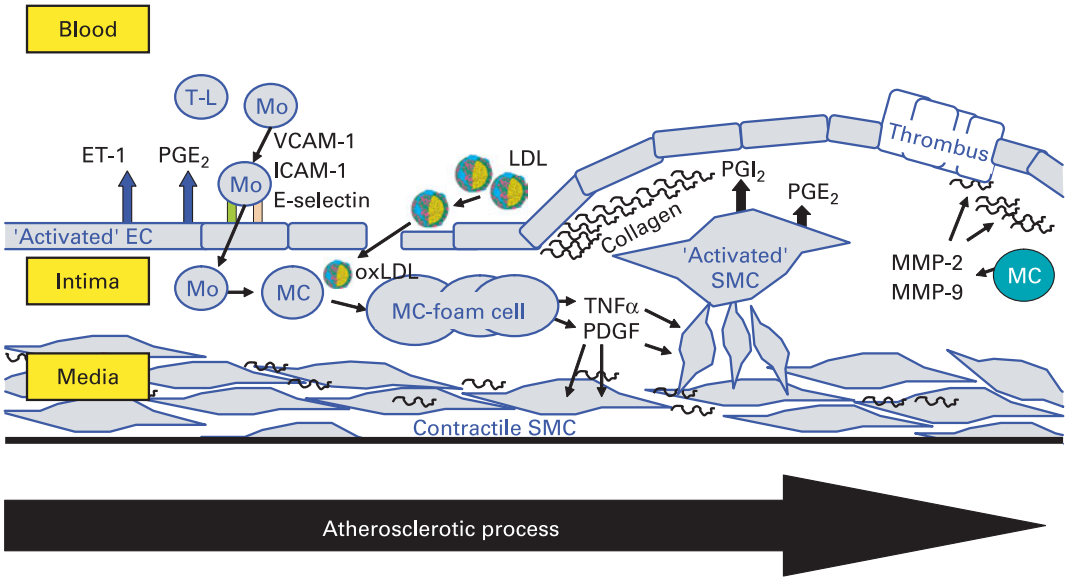

Fig. 2. Schematic presentation of the atherosclerotic process in the vascular wall showing the major cells contributing to atherosclerotic lesion development: endothelial cells (EC), smooth muscle cells (SMC) and monocyte-derived macrophages (MC). T-L, T-lymphocyte; Mo, monocyte; ET-1, endothelin-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; E-selectin, endothelial selectin; oxLDL, oxidatively modified LDL; MMP, matrix metalloproteinase; PDGF, plateled-derived growth factor.

British Journal of Nutrition

crucial factors for transcriptional induction of adhesion molecules and chemokines in endothelial cells[70], because the promoter region of endothelial selectin, ICAM-1, VCAM-1 and monocyte chemoattractant protein-1 contains multiple binding sites for NF-κB[71,72]. Hence, cytokines such as TNFα and many others, which are known activators of NF-κB, cause induction of endothelial cell adhesion molecules[70,73]. Due to their potential to bind and activate PPAR, CLA have been suggested to act in a similar manner as synthetic PPAR ligands and counter-regulate NF-κB-dependent transcription of adhesion molecules and chemokines through PPAR-dependent signalling. However, a recent study clearly demonstrated that the CLA isomers *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, at a concentration of 50 μM, which has to be regarded as a rather high concentration when considering physiological levels, do not modulate the cytokine-stimulated expression of adhesion molecules, monocyte adhesion and chemokine release as well as NF-κB activation in human aortic endothelial cells[74], although PPARγ was shown to be activated by CLA isomers in these cells. Since activation of PPARγ by *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, however, was only moderate in that study[74], it may be speculated that this is responsible for the lack of effect of CLA on leucocyte–endothelial cell interactions. In contrast to those findings in aortic endothelial cells, another study, indeed, revealed that 25 μM of either *cis*-9, *trans*-11-CLA or *trans*-10, *cis*-12-CLA decreases monocyte adhesion in human umbilical vein endothelial cells[75]. In the case of *cis*-9, *trans*-11-CLA, this incubation concentration is approximately in the range of the plasma concentration of this CLA isomer achieved in men consuming a CLA-rich diet which has been shown to be in range of 9·6 to 18 μM[76,77]. In contrast, the incubation concentration of *trans*-10, *cis*-12-CLA has to be regarded as quite high considering that the plasma concentration of *trans*-10, *cis*-12-CLA has been reported to be only 1·2 μM in men[78]. Investigations into the mechanisms involved revealed that the expressions of VCAM-1 and ICAM-1 were largely unaffected by both CLA isomers[75], which is similar to the findings in aortic endothelial cells[74]. However, through the use of platelet-activating factor (PAF) receptor antagonists and PAF synthesis inhibitors, it could be demonstrated that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA as well as a 50:50 mixture of these isomers inhibit cytokine-induced monocyte binding to umbilical vein endothelial cells by suppressing endothelial-generated PAF production[75]. Accordingly, it has been suggested that the action of CLA isomers in suppressing monocyte adhesion to umbilical vein endothelial cells is mediated through the attenuation of pro-inflammatory phospholipids such as PAF[75], which plays a central role in cytokine-induced monocyte adherence to the endothelium. The contradictory findings in aortic and umbilical vein endothelial cells, therefore, indicate that the effect of CLA isomers on monocyte adhesion is decisively influenced by the vascular origin of the endothelial cells used for experimentation. This, however, is not surprising since it is well established that vascular cells from different vascular beds as well as from different sections of the same blood vessel exert differential actions in response to a common stimulus[79,80]. In addition, cell type-specific effects of CLA are well documented in the literature[21,81,82]. Collectively, it cannot be unequivocally answered whether the anti-atherogenic effects of CLA observed in animal feeding experiments may be explained by modulating monocyte adhesion to the endothelium, which is a crucial step in the early phase of atherosclerosis. Based on the results in umbilical vein endothelial cells, it might be suggested that CLA exert their anti-atherogenic effects by attenuating adhesion of leucocytes to the endothelium (Fig. 3). Nevertheless, the observation from a randomised, placebo-controlled intervention trial that CLA supplementation with two different CLA mixtures (50:50 and 80:20 blends of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12 CLA) does not alter serum concentrations of soluble adhesion molecules in healthy volunteers[83], however, is supportive of the findings in aortic endothelial cells that CLA isomers, even at unphysiologically high concentrations, have no effect on cytokine-induced adhesion molecule expression, monocyte adhesion and chemokine release.

Conjugated linoleic acid effects on inflammatory mediator secretion from endothelial cells

Irrespective of direct influences on leucocyte–endothelial cell interactions, CLA might also exert atheroprotective effects through the modulation of the release of vasoactive mediators, which are involved in the regulation of vessel tone, blood pressure and inflammation. Several studies from independent groups demonstrated that CLA isomers such as *cis*-9, *trans*-11-CLA, *trans*-10, *cis*-12-CLA, *trans*-9, *trans*-11-CLA, and *cis*-9, *cis*-11-CLA as well as CLA isomeric mixtures, at concentrations of 2·6 to 50 μM, modulate the release of vasoactive substances including eicosanoids, NO and the potent vasoconstrictor endothelin-1 (ET-1) from endothelial cells[84–89]. These findings are of great importance with respect to the modulation of atherosclerosis development, since during endothelial dysfunction an abnormal vasoconstriction can be observed[90], which is attributed to an imbalance in the formation of vasoactive substances, in particular to increased levels of ET-1[91]. The pathogenetic relevance of increased ET-1 levels for atherosclerosis is demonstrated by the observation that ET-1 plasma levels strongly correlate with the carotid intima-media thickness in patients with increased risk of developing atherosclerosis[92]. Thus, the observation that treatment with 200 μM of a 50:50 mixture of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA decreased the release of ET-1 from human aortic endothelial cells[87] has to be considered beneficial with respect to maintaining vascular homeostasis, and, therefore, prevention of atherosclerosis. Synthetic PPAR agonists were also shown to suppress ET-1 secretion in cultured endothelial cells, by a mechanism involving inhibition of the AP-1 pathway[93,94]. Since CLA have been reported to significantly reduce activation of AP-1 in different cell types[95,96], it is not unlikely that the inhibitory effect of CLA on ET-1 release in endothelial cells is mediated by PPAR-dependent repression of AP-1. This, however, remains to be established.

Atheroprotective effects of CLA in endothelial cells might be also exerted by up-regulating intrinsic antioxidant enzymes, such as glutathione peroxidases, catalase and/or superoxide dismutase. Up-regulation of antioxidant defence mechanisms is of fundamental importance in protecting endothelial cells from oxidative damage[97], because oxidative damage is

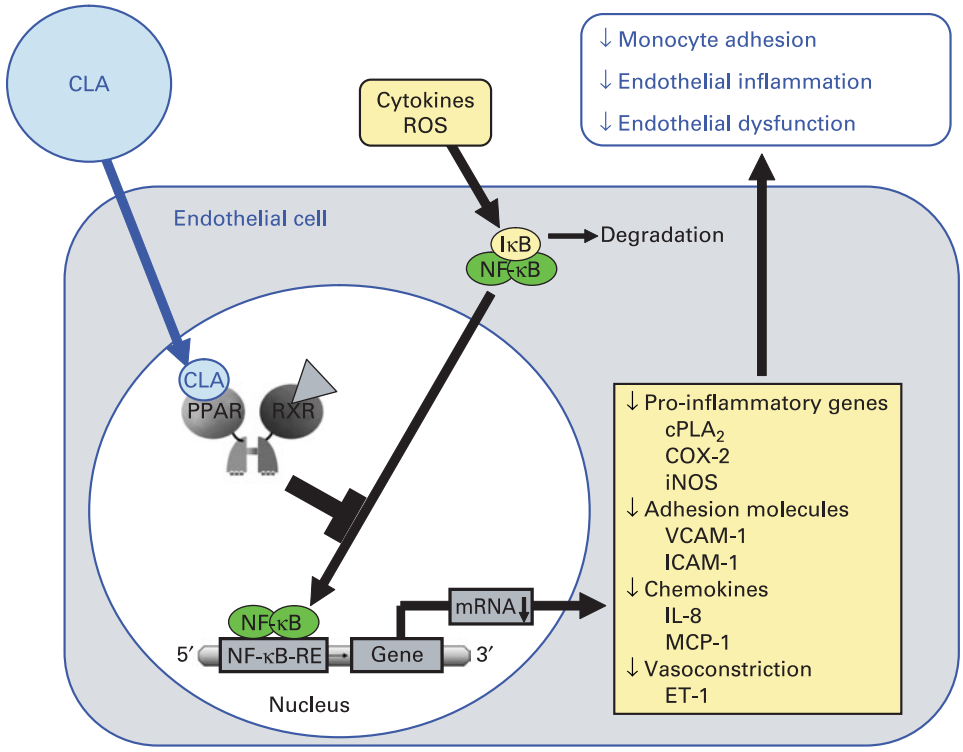

Fig. 3. Illustration of the effects of conjugated linoleic acid (CLA) on functional properties of endothelial cells. Through the activation of PPAR, CLA are capable of inhibiting NF-κB-regulated pro-inflammatory gene transcription leading to reduced monocyte–endothelial cell adhesion and endothelial inflammation. ROS, reactive oxygen species; IκB, inhibitor of κB; RXR, retinoid X receptor; $cPLA_2$, cytosolic phospholipase A_2; COX-2, cyclo-oxygenase-2; iNOS, inducible NO synthase; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; MCP-1; monocyte chemoattractant protein-1; RE, response element; ET-1, endothelin-1.

considered to be a major factor in the initiation of atherosclerotic lesions in the vascular wall[98,99]. In this regard it is, therefore, noteworthy that a recent study demonstrated that a 50:50 mixture of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, at a concentration of 30 to 90 μM, exhibited an inductive effect on gene expression of the redox enzyme glutathione peroxidase-4 in endothelial cells[100]. This effect of CLA on redox enzyme induction would be expected to enhance the capability of endothelial cells to attenuate oxidative and inflammatory stresses that are considered causal factors in coronary and other vascular diseases, and, thus, may explain, at least in part, the reported beneficial effects of CLA on vascular disease.

Influence of conjugated linoleic acids on smooth muscle cell function

Smooth muscle cells are predominant cellular components of atherosclerotic plaques and, thus, play an important role in the pathogenesis of atherosclerosis. In contrast to endothelial cells, which are involved, in particular, in the initial steps of atherosclerosis development, vascular smooth muscle cells play a dominant role during the progression of atherosclerosis, but also during restenosis after vascular interventions such as coronary angioplasty[101]. Smooth muscle cells have two different phenotypes[102]. The contractile or quiescent phenotype is characterised by the presence of typical smooth muscle cell contractile proteins (such as α-actin and smooth muscle myosin) and well-developed thick filaments. The main function of contractile smooth muscle cells, which dominate in the healthy vessel wall, where the smooth muscle cells are concentrically arranged in the arterial media and directly linked by cell contacts and connective tissue fibres, is contraction in response to chemical and mechanical stimuli, thereby maintaining vessel tone and regulating blood pressure. As a response to vascular injury, the contractile smooth muscle cells are activated and a change in smooth muscle cell phenotype is observed[103]. This phenotypic modulation is characterised by a dramatic change in smooth muscle cell morphology with a loss of contractile proteins and myofilaments and the formation of extensive rough endoplasmic reticulum and a large Golgi complex leading to a greatly increased synthetic, secretory and proliferative capacity, wherefore this phenotype is called the synthetic or activated one. Consequently, the synthetic smooth muscle cells migrate from the media into the arterial intima, where they proliferate and produce various substances including extracellular matrix (ECM) proteins and inflammatory mediators. The extensive production of ECM proteins including collagen, elastin, glycoproteins and proteoglycans by activated smooth muscle cells and accumulation of ECM proteins in the arterial wall significantly contribute to intimal thickening and, finally, atherosclerotic plaque formation. Collagen, in particular type I collagen, which accounts for 70 % of all collagen, is the dominating ECM protein making up to 60 % of the total protein content of atherosclerotic plaques[104,105]. Since vascular smooth muscle cells are the major source of collagen production within the vessel wall[106], collagen deposition

British Journal of Nutrition

by smooth muscle cells is considered to be a hallmark in atherosclerosis development. Activated smooth muscle cells, moreover, produce excessive amounts of inflammatory mediators including chemokines, cytokines and eicosanoids, which contribute to the chronic inflammatory response associated with atherosclerosis[107]. Thus, vascular smooth muscle cells are also strongly involved in maintaining the local inflammatory process in the arterial wall, besides activated endothelial cells and infiltrated leucocytes. Although events associated with phenotypic modulation (activation) of vascular smooth muscle cells, such as inflammatory mediator secretion and collagen formation by smooth muscle cells, are critical steps in atherosclerosis[108], only a few studies have addressed the impact of CLA isomers on either of these processes.

Conjugated linoleic acid effects on inflammatory mediator secretion from smooth muscle cells

Studies dealing with the action of CLA on inflammatory mediator secretion from activated smooth muscle cells clearly show that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA are capable of significantly reducing eicosanoid release from both resting and cytokine-activated vascular smooth muscle cells[109,110]. This observation is consistent with findings from studies in other cell-culture systems such as macrophages or endothelial cells[37,86,111], indicating that this effect of CLA isomers is independent of the cell type investigated. Investigations into the mechanisms responsible revealed that the reduced eicosanoid release from resting smooth muscle cells by CLA is probably the result of a reduced cellular pool of arachidonic acid (AA)[109,110], which serves as the main substrate for the biosynthesis of eicosanoids via cyclo-oxygenase (COX) enzymes. It has been reported that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA compete with other fatty acids such as linoleic acid for the incorporation into membrane phospholipids, but also that both CLA isomers interfere with the production of AA from linoleic acid[86,112], resulting in a reduced AA pool and, subsequently, reduced eicosanoid production. Noteworthy, the decline in AA levels in smooth muscle cell lipids was already observed at quite low concentrations of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA of about 5 μM[110], indicating that both CLA isomers effectively displace AA from membrane phospholipids and/or inhibit Δ5- and Δ6-desaturation of linoleic acid in smooth muscle cells. Regarding the CLA concentrations observed in the plasma of men consuming a CLA-rich diet (*cis*-9, *trans*-11-CLA: 9·6 to 18 μM[76,77], *trans*-10, *cis*-12-CLA: 1·2 μM[78]), these findings indicate that the effects observed in cultivated smooth muscle cells might also occur *in vivo*.

In addition to the reduction of AA levels in smooth muscle cell lipids by CLA isomers, the enzymic release of free AA from membrane phospholipids available for COX enzymes is probably also reduced by *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, as evidenced by the reduction of phospholipase A_2 (PLA_2) activity by both CLA isomers[86,113]. PLA_2 catalyses the release of AA from membrane phospholipids by hydrolysing the ester bonds at the *sn*-2 position of membrane phospholipids, where AA is mostly bound[114]. Since CLA isomers are also direct inhibitors of COX enzymes[115,116], the rate-limiting enzymes for prostanoid synthesis from AA, inhibition of these enzymes by CLA isomers in vascular smooth muscle cells might be also responsible for the observed reduction of prostanoid synthesis by CLA. Whether inhibition of COX activities is mediated by the CLA isomers themselves or their metabolites is unclear, because it has been shown that desaturation products of CLA isomers are also capable of inhibiting COX activities[115]. However, typical conjugated desaturation products of CLA isomers such as conjugated diene (CD) 20 : 3 or CD20 : 4, which are produced from CLA isomers by Δ6-desaturation, elongation and further Δ5-desaturation and detectable in hepatic tissues from CLA-fed animals[117,118], were not found at all in vascular smooth muscle cells and endothelial cells treated with CLA isomers[119–121], which is probably due to the very low fatty acid desaturation capacity of vascular smooth muscle cells and endothelial cells[122,123]. This would, therefore, suggest that desaturation products of CLA are not responsible for COX inhibition in smooth muscle cells. However, cells of the vessel wall such as smooth muscle cells and endothelial cells have a substantial capacity for the enzymic elongation of unsaturated fatty acids[122,123], which probably explains the detection of CLA isomer-specific elongation products such as CD20 : 2 (*cis*-11, *trans*-13) and CD20 : 2 (*trans*-12, *cis*-14) as well as CD22 : 2 (*cis*-13, *trans*-15) in smooth muscle cells and endothelial cells treated with 50 μM of either *cis*-9, *trans*-11-CLA or *trans*-10, *cis*-12-CLA[119,120]. Hence, it cannot be excluded that these elongation products of CLA may act as COX inhibitors, which, however, deserves further investigation.

Mechanistic studies in activated smooth muscle cells revealed that the reduction of cytokine-stimulated prostanoid release by *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA is mediated by a PPARγ-dependent inhibition of the NF-κB-pathway[109]. NF-κB is not only a central regulator of the expression of adhesion molecules and chemokines, but also of enzymes involved in the synthesis of prostanoids from AA, such as cytosolic PLA_2, COX-2 and microsomal PGE synthase (mPGES)[71]. This explains why increased expression of COX-2, mPGES and cytosolic PLA_2, which are co-localised to the endoplasmic reticulum and nuclear envelope[124,125], by NF-κB activators, such as cytokines, results in the excessive formation of prostanoids[126]. Hence, the observation that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA at a concentration of 50 μM, which is slightly above the physiological concentration range, caused a marked inhibition of cytokine-induced expression of cytosolic PLA_2, COX-2 and mPGES in vascular smooth muscle cells probably largely explains the reduction of prostanoid release from activated smooth muscle cells. Inhibition of NF-κB activation and, subsequently, reduced expression of genes involved in prostanoid synthesis as well as other inflammatory genes has also been demonstrated to be causative for the reduced expression of inflammatory genes in vascular smooth muscle cells treated with pharmacological PPARγ ligands[127–130]. This indicates that CLA isomers have similar properties as pharmacological PPARγ ligands with respect to modulating prostanoid release from activated smooth muscle cells. Because excessive formation of inflammatory mediators by smooth muscle cells contributes to atherosclerotic plaque

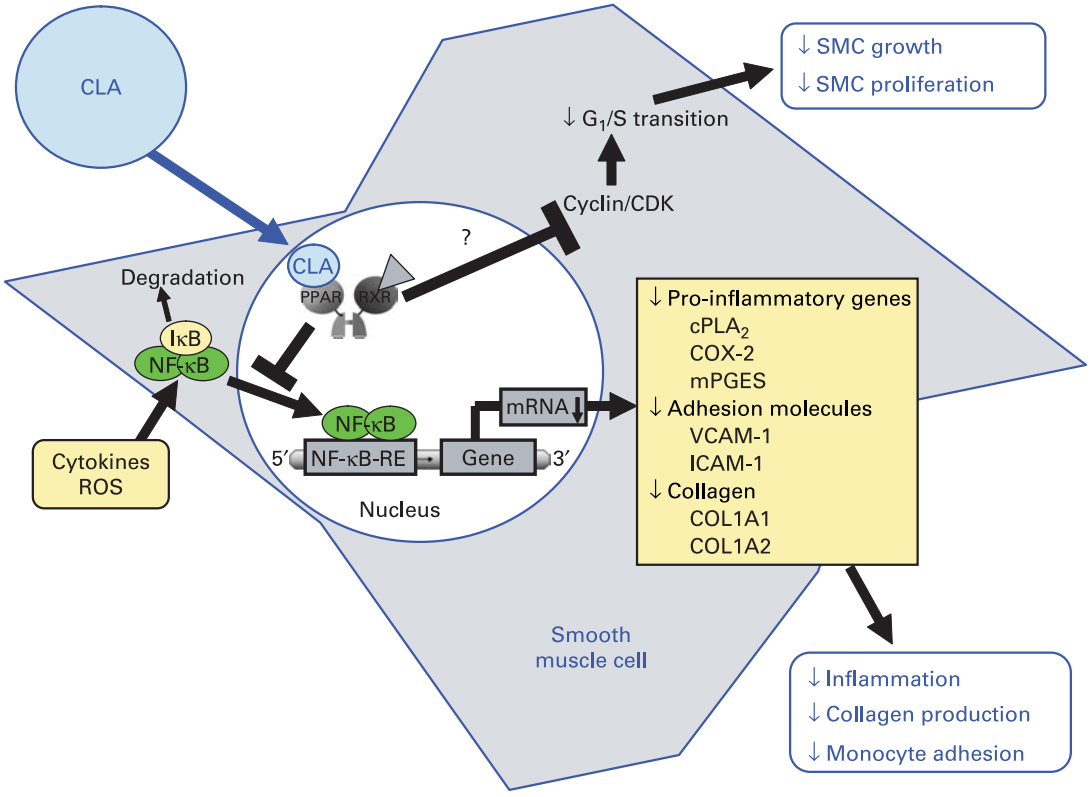

Fig. 4. Illustration of the effects of conjugated linoleic acid (CLA) on functional properties of smooth muscle cells (SMC). Through the activation of PPAR, CLA are capable of inhibiting NF-κB-regulated pro-inflammatory gene transcription leading to reduced collagen production, inflammatory mediator secretion and monocyte recruitment. Whether CLA also inhibit smooth muscle cell growth and proliferation by blocking G_1/S cell cycle transition through the induction of cyclin-dependent kinase (CDK) inhibitors remains to be established. RXR, retinoid X receptor; IκB, inhibitor of κB; $cPLA_2$, cytosolic phospholipase A_2; COX-2, cyclo-oxygenase-2; mPGES, microsomal PGE synthase; ROS, reactive oxygen species; RE, response element; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; COL, collagen.

development, the findings in smooth muscle cells suggest that the anti-inflammatory action of CLA is, at least partially, responsible for the anti-atherogenic effects of CLA observed *in vivo* (Fig. 4).

A recent study further revealed that CLA inhibits cytokine-induced expression of the adhesion molecules ICAM-1 and VCAM-1 in vascular smooth muscle cells[131]. Curiously, only 100 μM of a 50:50 mixture of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA elicited a significant attenuation of expression of ICAM-1 and VCAM-1, whereas the individual isomers were ineffective. Although the reason for this surprising result is unclear, the observed attenuation of adhesion molecule expression by the CLA mixture might be of relevance when trying to explain the anti-atherogenic effects of CLA *in vivo*, because increased adhesion molecule expression in vascular smooth muscle cells is also involved in the recruitment of monocytes into sites of vascular injury, and contributes to the inflammatory process in the vascular wall and plaque progression[132,133].

Conjugated linoleic acid effects on smooth muscle cell collagen production

Regarding an influence of CLA on the production of ECM proteins by vascular smooth muscle cells, which is a critical event of atherosclerosis development, a recent study revealed that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, at a concentration of 50 μM, are capable of inhibiting the production of collagen by activated smooth muscle cells[134], by a mechanism probably involving PPARγ-mediated inhibition of NF-κB. This is based on the finding that simultaneous treatment of smooth muscle cells with a PPARγ-specific antagonist abrogated the inhibitory effect of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA on smooth muscle cell collagen formation and NF-κB activation, and that different agents such as AA and oxidised LDL stimulate collagen production in vascular smooth muscle cells via activation of NF-κB[135,136]. Stimulation of collagen production via activation of NF-κB is explained by the fact that the promoter of the collagen (COL) 1A2 gene, which encodes the α2 chain of type I collagen, contains at least two putative NF-κB binding sites[137]. In addition, the COL1A1 gene, which encodes the α1 chain of type I collagen, is probably also induced by NF-κB, because both COL1A1 and COL1A2 are highly sensitive to reactive oxygen species[138,139], which are major factors inducing the phosphorylation of the inhibitors of NF-κB and subsequent translocation of NF-κB to the nucleus[140]. Furthermore, in line with our assumption that NF-κB inhibition is causative for the reduced collagen formation by CLA isomers, is the finding that inhibition of NF-κB activity by the antioxidant (−)-epigallocatechin-3-gallate in activated hepatic stellate cells is accompanied by a reduced collagen production[141]. Moreover, the PPARγ activator 15-deoxy-$\Delta^{12,14}$-PGJ_2, which reduced collagen formation in human

British Journal of Nutrition

aortic smooth muscle cells[142], could be also demonstrated to inhibit NF-κB[143]. A further mechanism contributing to the reduction of collagen production by *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA might be the above-mentioned inhibition of AA metabolism by COX and lipoxygenase enzymes to biologically active eicosanoids[109,110], because these metabolites are supposed to be mediators of pathological fibrotic conditions increasing the formation of collagen by stimulating pro-fibrotic factors such as transforming growth factor (TGF)-β1[144]. Hence, antagonism of specific eicosanoid receptors or selective inhibition of COX-2 results in attenuation of fibrosis under different pathological conditions, which is accompanied by a decrease in the formation of TGF-β1[145–147]. Thus, future studies have to clarify whether inhibition of AA metabolism, besides PPARγ activation, might also contribute to the reduced smooth muscle cell collagen formation in response to *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA. Since deposition of collagen and other ECM proteins in the vessel wall largely contributes to the formation of atherosclerotic plaques, the reduced collagen production by vascular smooth muscle cells treated with CLA might explain, at least in part, the anti-atherogenic effects of CLA.

Since smooth muscle cell migration and proliferation precedes smooth muscle cell production of ECM proteins, it would be interesting to know whether CLA might also affect smooth muscle cell proliferation, which has not yet been investigated. In this context, it is worth mentioning that PPARα and PPARγ agonists inhibit smooth muscle cell proliferation by blocking G_1/S cell cycle transition through the induction of cyclin-dependent kinase inhibitors[148–150], leading to smooth muscle cell growth inhibition and reduced neointima formation[148]. Considering that both CLA isomers, such as *trans*-10, *cis*-12-CLA, and CLA mixtures cause the inhibition of proliferation of numerous cell types by a mechanism involving inhibition of G_1/S cell cycle progression[151–154], inhibition of smooth muscle cell proliferation by CLA would be not unexpected. In endothelial cells, CLA isomeric mixtures or pure CLA isomers were also demonstrated to inhibit cell proliferation[155,156]. The biological importance of this antiproliferative effect, which involves activation of the proapoptotic caspase-3[156], in endothelial cells, however, is less clear because the proliferation rate of endothelial cells is low in healthy vessels and the significance of endothelial cell proliferation for the development of atherosclerosis is still discussed controversially. Whereas an increased proliferation rate could be interpreted as beneficial in view of re-endothelialisation after endothelial microdamage[157], it could be shown that anti-proliferative effects are beneficial in view of prevention from plaque development[158].

Influence of conjugated linoleic acids on macrophage function

In addition to endothelial cells and smooth muscle cells, monocyte-derived macrophages are important contributors to the development of atherosclerotic plaques. During early atherosclerosis, monocytes are recruited into the subendothelial space of the artery wall, where they differentiate into macrophages. During differentiation into macrophages, monocyte-derived macrophages secrete a large number of chemotactic substances and growth factors[159], which promote the infiltration of the vascular wall by further leucocytes as well as the migration and proliferation of smooth muscle cells. Monocyte-derived macrophages, moreover, induce the oxidative modification of LDL, which is also considered a key event in the early pathogenesis of atherosclerosis, leading to an enhanced uptake of chemically modified LDL into the macrophages by way of scavenger receptors, such as CD36 and scavenger receptor-A, highly expressed on the plasma membrane of differentiated macrophages[160]. Since the expression of scavenger receptors is not under negative feedback control by cellular cholesterol content, the LDL-derived lipids, such as cholesterol and TAG, accumulate within the macrophage, leading to a foamy appearance of the cytoplasm, wherefore these cells are called foam cells[161]. The transformation of macrophages into foam cells is a critical step in the atherosclerotic process, since their accumulation in the arterial wall leads to the formation of fatty streaks, which are the first visible atherosclerotic lesions.

Conjugated linoleic acid effects on inflammatory mediator secretion from monocyte-derived macrophages

Several studies have been performed investigating the modulatory potential of CLA isomers on inflammatory mediator secretion from macrophages. The vast majority of these studies revealed that the CLA isomers *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA or mixtures of both CLA isomers, at concentrations of 25 to 200 μM, inhibit the expression of pro-inflammatory genes such as cytosolic PLA_2, COX-2, inducible NO synthase and TNFα, thereby decreasing the release of inflammatory mediators including PGE_2, NO, TNFα as well as IL-1 and IL-6[37,111,113,162–166]. In contrast to these findings, two studies in RAW264.7 macrophages[167] and porcine peripheral blood polymorphonuclear cells[168], respectively, found an increased expression of TNFα following treatment with *trans*-10, *cis*-12-CLA. The reasons underlying these contradictory observations between the aforementioned and the latter two studies, however, remain unresolved. The anti-inflammatory properties of CLA isomers in macrophages were shown to be mediated, at least in part, by a PPARγ-dependent inhibition of NF-κB[37,111], which regulates a large number of inflammatory genes involved in the synthesis of inflammatory mediators. PPARγ-dependent inhibition of NF-κB has also been shown to be responsible for the inhibitory effect of CLA isomers on inflammatory mediator release from activated smooth muscle cells[109] and bronchial epithelial cells[22], which indicates that this effect of CLA is largely independent of the cell type used. Since synthetic PPARγ agonists were also shown to exert anti-inflammatory effects in macrophages by inducing the expression of anti-inflammatory genes, such as the IL-1 receptor antagonist[169], future studies have to clarify whether CLA might also influence this pathway of inflammation control. A further, alternative pathway of inflammation control that might be elicited by CLA in macrophages could be modulating the activation and differentiation of macrophages. In fact, PPARγ agonists have been demonstrated to enhance the formation of a sub-population of 'alternatively activated' (M2) macrophages[170,171], which display a more pronounced anti-inflammatory phenotype compared with 'classically activated' (M1) macrophages. This effect of PPARγ agonists is mediated by inhibition of the transcription

British Journal of Nutrition

factors AP-1 and signal transducers and activators of transcription (STAT)-1, both of which are involved in the induction of pro-inflammatory cytokines during M1 differentiation[172,173]. Whether CLA are capable of modulating macrophage activation, however, remains to be established. In addition to PPARγ, CLA have been demonstrated to activate PPARα and PPARδ[35–37,174], both of which are known to mediate anti-inflammatory effects by negatively interfering with pro-inflammatory signalling pathways, such as NF-κB and AP-1[175–177]. Therefore, it is not unlikely that the anti-inflammatory effects of CLA in macrophages are also mediated by the activation of other PPAR isotypes, namely PPARα and PPARδ.

In view of the fact that pro-inflammatory molecules such as TNFα, IL-1 and IL-6 are known to promote endothelial cell inflammation, monocyte differentiation into macrophages and smooth muscle cell proliferation, and thereby a chronic, progressive inflammatory process of the arterial wall which is characteristic of atherosclerosis, the inhibitory effect of CLA on the secretion of TNFα, IL-1 and IL-6 from macrophages has to be regarded as beneficial with respect to protection from atherosclerosis (Fig. 5).

Conjugated linoleic acid effects on macrophage cholesterol homeostasis

Besides modulating inflammatory processes, *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, at a concentration of 50 μM, have also recently been shown to influence cholesterol accumulation in macrophage-derived foam cells[178]. Both CLA isomers were shown to reduce cholesterol accumulation as evidenced by lowered concentrations of total and esterified cholesterol, the storage form of cholesterol in macrophage-derived foam cells, and to stimulate HDL-dependent cholesterol efflux in RAW264.7 macrophage-derived foam cells[178]. This effect, however, is cell type-specific because in human THP-1 macrophage-derived foam cells treatment with *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, at a concentration of 100 μM, failed to reduce cholesterol accumulation[179]. The reason for this cell type-specific effect of CLA cannot be definitely explained, but differences in the cellular uptake and incorporation of CLA isomers between the two cell types might be causative. Whereas in THP-1 macrophages only a poor incorporation of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA in total cell lipids after treatment with CLA isomers has been reported[179], treatment of RAW264.7 macrophage-derived foam cells with *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA resulted in a marked incorporation of CLA isomers[178]. Since synthetic activators of PPARα and PPARγ also stimulate cholesterol removal from macrophage-derived foam cells[180–184], it has been postulated that the effect of CLA isomers, as natural PPAR ligands, on RAW264.7 macrophage cholesterol accumulation is also mediated by a PPAR-dependent mechanism[178]. Indeed, similarly as observed with synthetic PPARα and PPARγ activators[180–184], *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA also cause the up-regulation of several genes involved in cholesterol homeostasis in macrophage-derived foam cells such as liver X receptor-α (LXRα), ATP-binding cassette (ABC) A1, Niemann-Pick-C1 (NPC-1) and NPC-2[178]. Increased expression and activation of the transcription factor LXRα leads to a reduction in cholesterol accumulation, because LXRα activates the transcription of the cholesterol exporters ABCA1 and ABCG1[180,182,185]. ABCA1 in

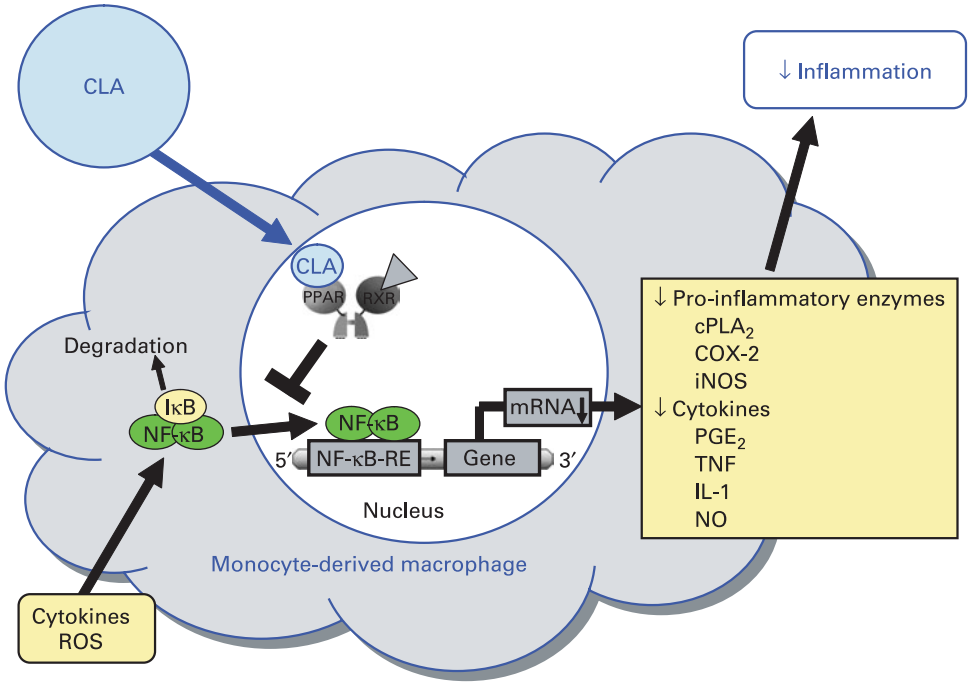

Fig. 5. Illustration of the effects of conjugated linoleic acid (CLA) on functional properties of monocyte-derived macrophages. Through the activation of PPAR, CLA are capable of inhibiting NF-κB-regulated pro-inflammatory gene transcription leading to reduced inflammatory mediator secretion from macrophages. RXR, retinoid X receptor; $cPLA_2$, cytosolic phospholipase A_2; COX-2, cyclo-oxygenase-2; iNOS, inducible NO synthase; IκB, inhibitor of κB; RE, response element; ROS, reactive oxygen species.

particular plays a key role in cellular cholesterol efflux from macrophages to extracellular acceptors such as apo-AI, the first step in reverse cholesterol transport, which is responsible for cholesterol transport from peripheral tissues to the liver. The essential role of ABCA1 for cholesterol efflux has been demonstrated in patients with a mutated ABCA1 gene[186–188], where cholesterol efflux and reverse cholesterol transport are impaired. As a consequence of induction of ABCA1 and ABCG1 by PPARα and PPARγ activators, the apo-AI- and HDL-dependent cholesterol efflux in macrophages is enhanced[180,182,185]. Up-regulation of ABCG1 by CLA isomers in macrophage-derived foam cells might also contribute to increased cholesterol removal. This suggestion is supported by findings in macrophages, where CLA isomers, at a concentration of 100 μM, were shown to stimulate ABCG1 expression by a mechanism involving sterol regulatory element binding protein-1c[189]. Furthermore, *cis*-9, *trans*-11-CLA- and *trans*-10, *cis*-12-CLA-induced up-regulation of NPC-1 and NPC-2 probably also contributes to the lowering of cholesterol accumulation in RAW264.7 macrophage-derived foam cells[178], since both proteins mediate intracellular transport of cholesterol from the late endosomal compartment and lysosome, respectively, to the plasma membrane[190,191], thereby increasing the availability of cholesterol at the cell membrane for efflux through extracellular acceptors such as HDL or apo-AI. Whether CLA isomers might also influence genes regulating cholesterol esterification in macrophage-derived foam cells such as acyl-CoA:cholesterol acyltransferase (ACAT), which catalyses cholesteryl ester formation from cholesterol and fatty acyl-CoA, and cholesteryl ester hydrolase, which is responsible for the hydrolysis of stored cholesteryl esters in macrophage-derived foam cells and release of non-esterified cholesterol for HDL-mediated efflux, is currently unknown. However, two independent groups[181,192] have demonstrated that the reduction of cholesteryl ester accumulation by pharmacological PPARα and PPARγ ligands in macrophages was accompanied by enhanced cholesteryl ester hydrolase mRNA expression and inhibited ACAT-1 mRNA expression. Thus, down-regulation of ACAT-1 and up-regulation of cholesteryl ester hydrolase by CLA isomers could also contribute to the reduced cholesteryl ester concentrations as observed in RAW264.7 macrophage-derived foam cells[178]. This, however, deserves future investigation. Collectively, the data from studies with macrophages and macrophage-derived foam cells[178,189] suggest that reverse cholesterol transport is stimulated by CLA isomers. This suggestion is also supported by the observation from an *in vivo* study showing that plasma HDL-cholesterol concentrations and ABCA1 gene expression are increased in the aorta of hamsters fed *cis*-9, *trans*-11-CLA[193]. Because excessive accumulation of cholesterol by macrophage-derived foam cells in the arterial wall leads to atherosclerosis, the recent findings[178,189] in connection with other beneficial effects of CLA in macrophages[113,140,163] might also in part explain the anti-atherogenic actions of CLA (Fig. 6).

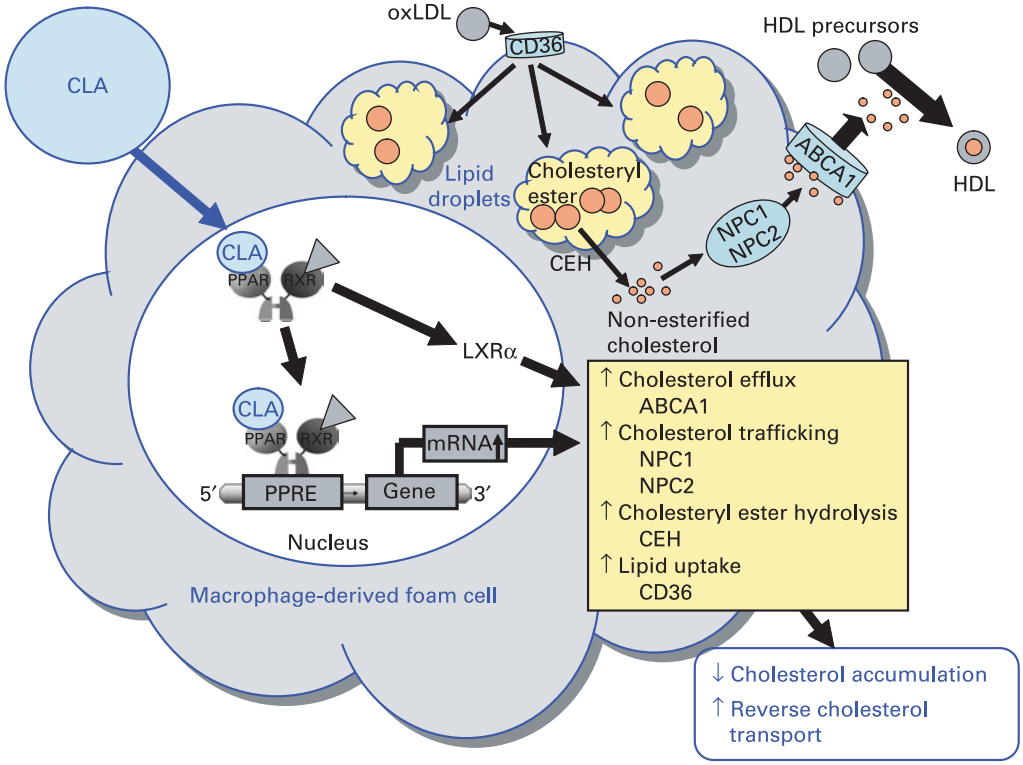

Fig. 6. Illustration of the effects of conjugated linoleic acid (CLA) on functional properties of macrophage-derived foam cells. Through the activation of PPAR, CLA exert stimulatory effects on the transcription of genes involved in cholesteryl ester hydrolysis, intracellular cholesterol trafficking and cholesterol efflux leading to a reduced cholesterol accumulation and a stimulated reverse cholesterol transport. oxLDL, oxidatively modified LDL; ABCA1, ATP-binding cassette transporter A1; NPC1/2, Niemann-Pick-C1/2; CEH, cholesteryl ester hydrolase; RXR, retinoid X receptor; LXRα, liver X receptor α; PPRE, peroxisome proliferator response element.

Conjugated linoleic acid effects on matrix-metalloproteinase-associated extracellular matrix degradation

Pathological studies have shown that rupture-prone areas such as the shoulder region of atherosclerotic plaques are frequently infiltrated with a large number of monocyte-derived macrophages[194]. These plaque-associated macrophages produce large quantities of matrix-metalloproteinases (MMP), particularly MMP-9 and MMP-2[195–197], which participate in ECM degradation and destabilisation of plaques[198–200], thereby promoting acute cardiovascular events such as myocardial infarction and stroke, which are typical late-stage events of atherosclerosis. In addition, ECM degradation by MMP also enables medial smooth muscle cells to migrate into the intima, where they proliferate and promote atherosclerosis development. Elevated blood levels of MMP in patients with coronary artery disease clearly indicate their important role in the atherosclerotic process[201,202]. Irrespective of their great importance for atherosclerosis, only one published study has investigated the impact of CLA, *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, on macrophage MMP expression and activity[203]. According to this study in THP-1 macrophages[203], CLA isomers, at the concentrations of 10 and 100 μM, are not able to reduce phorbol myristate acetate-induced gene expression and gelatinolytic activity of MMP-2 and MMP-9 and to alter gene expression of tissue inhibitors of metalloproteinases (TIMP)-1 and TIMP-2, which are critical for the regulation of MMP activity in macrophages[204,205]. In contrast, the synthetic PPARγ ligand troglitazone significantly reduced gene expression and activity of both MMP in that study[203], which is consistent with findings from other studies using synthetic PPARγ agonists[172,206]. Since PPARγ-mediated repression of NF-κB, which is an important transcriptional regulator of MMP-9 and MMP-2[207–210], largely constitutes the mechanistic basis for diminished MMP expression by synthetic PPARγ ligands in macrophages[172,206], the lack of effect of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA in THP-1 macrophages is probably explained by the observation that both CLA isomers, in contrast to troglitazone, neither activated PPARγ nor reduced DNA-binding activity of NF-κB[203]. One reason possibly explaining the failure of treatment with *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA might be the fact that CLA isomers are comparatively weak PPARγ ligands due to a low binding affinity and, therefore, cause only a weak PPARγ transactivation[203]. In contrast, troglitazone has a high affinity for PPARγ as evidenced by ligand-binding assays[211]. The lower binding affinity of CLA isomers for PPARγ compared with troglitazone might be of decisive importance in the THP-1 macrophage cell model, because other PPAR subtypes such as PPARα and PPARβ/δ are also highly expressed in THP-1 cells[169], and CLA binds to and activates all PPAR subtypes with similar efficiency[36,133]. Therefore, it might be speculated that the low PPARγ activation by *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA in THP-1 cells is due to a competition of the various PPAR subtypes for binding of CLA isomers to their ligand-binding domains. Thus, the effect of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA on PPARγ would be expected to be higher in a cell type expressing predominantly the PPARγ subtype. Indeed, a recent study demonstrated that several CLA isomers caused a pronounced activation of PPARγ in RAW264.7 macrophage cells[37], which predominantly express PPARγ, whereas neither PPARα nor PPARβ/δ was detectable. Irrespective of this, the available data suggest that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA are ineffective in macrophage MMP-associated ECM degradation, which contributes to the progression and rupture of advanced atherosclerotic plaques in the late stage of atherosclerosis. It, moreover, suggests that *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA may exert their anti-atherogenic actions by other mechanisms or during earlier stages of atherosclerosis. Nevertheless, future studies have to clarify whether CLA are capable of modulating MMP secretion and activity in other macrophage cell models, for example, in RAW264.7 macrophages, or in vascular smooth muscle cells. In vascular smooth muscle cells, activation of PPARγ inhibited MMP-9 mRNA and protein expression, gelatinolytic activity as well as migration of vascular smooth muscle cells[128]. The latter is explained by a reduced movement of smooth muscle cells, which are embedded in the interstitial ECM, as a consequence of the diminished gelatinolytic activity. Since ECM degradation by MMP and medial smooth muscle cell movement into the intima significantly contribute to atherosclerotic plaque development, a potential inhibition of MMP secretion and activity by CLA in vascular smooth muscle cells would have important implications for atherosclerosis and might also contribute to the potent anti-atherogenic effects of CLA.

Conclusions

Based on *in vitro* studies dealing with the effects of CLA isomers and CLA mixtures on functional properties of vascular cells, it can be concluded that CLA exert several beneficial actions in cells of the arterial wall through the activation of nuclear PPAR. These actions of CLA, which may, at least partially, explain the inhibition of atherogenesis by dietary CLA, include modulation of vasoactive mediator release from endothelial cells, inhibition of inflammatory and fibrotic processes in activated smooth muscle cells, abrogation of inflammatory responses in activated macrophages, and reduction of cholesterol accumulation in macrophage-derived foam cells. Whether other mechanisms than activation of PPAR are also involved in the mediation of these actions of CLA cannot be ruled out. However, it is very likely that some of the effects of CLA such as reduction of eicosanoid release from endothelial cells and smooth muscle cells under resting conditions are mediated by competition of CLA with other fatty acids for the incorporation into membrane phospholipids but also due to interference with enzymes involved in Δ5- and Δ6-desaturation. With respect to the mediation of the anti-atherogenic effects of CLA, the metabolites of CLA should be taken into account as well. This is based on the observation that metabolites of CLA exhibit a strong biological activity *in vitro*, and are formed in vascular cells treated with CLA isomers in significant amounts.

Although isomer-specific effects of CLA are well documented in the literature[81,82], there is only little evidence for isomer-specific effects of CLA in cells of the vascular wall. Only two studies in macrophages revealed a pro-inflammatory effect for the *trans*-10, *cis*-12-CLA isomer[167,168], which could, however, not be observed in endothelial cells or

smooth muscle cells. In addition, there are also reports that did not observe a pro-inflammatory effect of the *trans*-10, *cis*-12-CLA isomer in macrophages[37,113]. Hence, the majority of data from studies dealing with the effects of CLA in vascular cells suggest that the effects of CLA on processes related to the development of atherosclerosis are largely independent of structural differences (position and geometry of the double bonds) between the individual CLA isomers. This suggestion is also in accordance with obervations from *in vivo* experiments with rabbits and hamsters where different CLA isomers and CLA mixtures seem to have similar effects on atherosclerosis development[18,212–215]. Nevertheless, in mouse models of atherosclerosis there is evidence for opposing effects of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA on atherosclerotic plaque formation[216]. Namely, the *cis*-9, *trans*-11-CLA isomer significantly reduced the cross-sectional lesion area of the aortic root, whereas the *trans*-10, *cis*-12-CLA isomer significantly increased lesion area compared with control. Moreover, *en face* examination of the aorta of *trans*-10, *cis*-12-CLA-fed mice revealed an increased lesion area in specific regions of the vessel and suggested that the *trans*-10, *cis*-12-CLA isomer induced a pro-oxidative state[203]. Due to these pro-atherogenic effects observed with *trans*-10, *cis*-12-CLA, but also other detrimental effects of *trans*-10, *cis*-12-CLA reported in the literature (lipid peroxidation, decrease in insulin sensitivity, lowering of HDL-cholesterol)[31,32,34,217–220], the uptake of dietary CLA supplements, which usually have a high content of *trans*-10, *cis*-12-CLA, should generally be considered critically. Nevertheless, future studies have to clarify why the two most frequently studied CLA isomers, *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, act differentially in *in vivo* studies with human subjects and mice but not in *in vitro* studies with cells of the vascular wall.

Although a definite reason for cell type-specific effects of CLA (i.e. THP-1 *v.* RAW264.7) cannot be given, it might be speculated that differences in the cellular uptake and incorporation of CLA isomers between different cell types are causative. The comparable effects of CLA isomers on the production of inflammatory mediators in endothelial cells and smooth muscle cells might, therefore, be explained by a similar efficiency for the uptake and incorporation of CLA in these two cell types as evidenced by similar concentrations of CLA isomers in total lipids of endothelial cells and smooth muscle cells following treatment with CLA isomers. Accordingly, the divergent effects of CLA on molecular markers of cholesterol homeostasis in macrophages (lack of effect in THP-1 cells, pronounced effect in RAW264.7 cells) is probably due to different incorporation rates of CLA isomers into macrophage cell lipids (poor incorporation in THP-1 cells, marked incorporation in RAW264.7 cells). Nevertheless, other reasons such as differences in the expression pattern of PPAR isotypes between these two cell lines might also apply. Whereas THP-1 cells express all PPAR isotypes[169], RAW264.7 cells predominantly express the PPARγ isotype[37]. Since CLA bind to and activate all PPAR isotypes with similar efficiency[20,35,36], it might be speculated that the low PPARγ activation by CLA isomers in THP-1 cells[203] is due to a competition of the various PPAR isotypes for binding of CLA isomers to their ligand-binding domains. Thus, an effect of CLA on PPARγ would be expected to be higher in a cell type expressing predominantly the PPARγ isotype such as RAW264.7 cells. In line with this assumption is the observation that CLA isomers including *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA cause a pronounced activation of PPARγ in RAW264.7 cells[37]. Nevertheless, to resolve the reason for the cell type-specific effects of CLA with certainty, further research is required.

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References

1. Sehat N, Kramer JK, Mossoba MM, *et al.* (1998) Identification of conjugated linoleic acid (CLA) isomers in cheese by gas chromatography, silver ion high performance liquid chromatography, and mass spectral reconstructed ion profiles: comparison of chromatographic elution sequences. *Lipids* **33**, 963–971.
2. Steinhart H, Rickert R & Winkler K (2003) Identification and analysis of conjugated linoleic acid isomers (CLA). *Eur J Med Res* **8**, 370–372.
3. Jahreis G & Kraft J (2002) Sources of conjugated linoleic acid in the human diet. *Lipid Technol* **14**, 29–32.
4. Chin SF, Liu W, Storkson JM, *et al.* (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J Food Compos Anal* **5**, 185–197.
5. Griinari JM, Corl BA, Lacy SH, *et al.* (2000) Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ(9)-desaturase. *J Nutr* **130**, 2285–2291.
6. Palmquist DL, St-Pierre N & McClure KE (2004) Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. *J Nutr* **134**, 2407–2414.
7. Corl BA, Baumgard LH, Dwyer DA, *et al.* (2001) The role of Δ(9)-desaturase in the production of *cis*-9, *trans*-11 CLA. *J Nutr Biochem* **12**, 622–630.
8. Fritsche J & Steinhart H (1998) Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. *Z Lebensm Unters Forsch A* **206**, 77–82.
9. Kuhnt K, Kraft J, Moeckel P, *et al.* (2006) *Trans*-11-18 : 1 is effectively Δ9-desaturated compared with *trans*-12-18 : 1 in humans. *Br J Nutr* **95**, 752–761.
10. Kuhnt K, Kraft J, Vogelsang H, *et al.* (2007) Dietary supplementation with *trans*-11- and *trans*-12-18 : 1 increases *cis*-9,*trans*-11-conjugated linoleic acid in human immune cells, but without effects on biomarkers of immune function and inflammation. *Br J Nutr* **97**, 1196–1205.
11. Wolff RL & Precht D (2002) Reassessment of the contribution of bovine milk fats to the *trans*-18 : 1 isomeric acid consumption by European populations. Additional data for rumenic (*cis*-9,*trans*-11 18 : 2) acid. *Lipids* **37**, 1149–1150.
12. Martins SV, Lopes PA, Alfaia CM, *et al.* (2007) Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal. *Br J Nutr* **98**, 1206–1213.

13. Jiang J, Wolk A & Vessby B (1999) Relation between the intake on milk fat and the occurrence of conjugated linoleic acid in human adipose tissue. *Am J Clin Nutr* **70**, 21–27.
14. Ritzenthaler KL, McGuire MK, Falen R, *et al.* (2001) Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. *J Nutr* **131**, 1548–1554.
15. Herbel BK, McGuire MK, McGuire MA, *et al.* (1998) Safflower oil consumption does not increase plasma conjugated linoleic acid concentrations in humans. *Am J Clin Nutr* **67**, 332–337.
16. Bhattacharya A, Banu J, Rahman M, *et al.* (2006) Biological effects of conjugated linoleic acids in health and disease. *J Nutr Biochem* **17**, 789–810.
17. Ha YL, Grimm NK & Pariza MW (1987) Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* **8**, 1881–1887.
18. Lee KN, Kritchevsky D & Pariza MW (1994) Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **108**, 19–25.
19. Cook ME, Miller CC, Park Y, *et al.* (1993) Immune modulation by altered nutrient metabolism: nutritonal control of immune-induced growth depression. *Poult Sci* **72**, 1301–1305.
20. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, *et al.* (1998) Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. *Biochem Biophys Res Commun* **244**, 678–682.
21. Park Y, Storkson JM, Albright KJ, *et al.* (1999) Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* **34**, 235–241.
22. Jaudszus A, Krokowski M, Möckel P, *et al.* (2008) *Cis*-9, *trans*-11-conjugated linoleic acid inhibits allergic sensitization and airway inflammation via a PPARγ-related mechanism in mice. *J Nutr* **138**, 1336–1342.
23. Rosamond W, Flegal K, Furie K, *et al.* (2008) Heart disease and stroke statistics – 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* **117**, e25–e146.
24. Ross R (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **362**, 801–809.
25. Mitchell PL & McLeod RS (2008) Conjugated linoleic acid and atherosclerosis: studies in animal models. *Biochem Cell Biol* **86**, 293–301.
26. Nakamura YK, Flintoff-Dye N & Omaye ST (2008) Conjugated linoleic acid modulation of risk factors associated with atherosclerosis. *Nutr Metab (Lond)* **5**, 22.
27. Benito P, Nelson GJ, Kelley DS, *et al.* (2001) The effect of conjugated linoleic acid on plasma lipoproteins and tissue fatty acid composition in humans. *Lipids* **36**, 229–236.
28. Riserus U, Berglund L & Vessby B (2001) Conjugated linoleic acid (CLA) reduces abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial. *Int J Obes* **25**, 1129–1135.
29. Petridou A, Mougios V & Sagredos A (2003) Supplementation with CLA: isomer incorporation into serum lipids and effect on body fat of women. *Lipids* **38**, 805–811.
30. Smedman A & Vessby B (2001) Conjugated linoleic acid supplementation in humans – metabolic effects. *Lipids* **36**, 773–781.
31. Risérus U, Basu S, Jovinge S, *et al.* (2002) Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid induced insulin resistance. *Circulation* **106**, 1925–1929.
32. Tricon S, Burdge GC, Kew S, *et al.* (2004) Opposing effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid on blood lipids in healthy humans. *Am J Clin Nutr* **80**, 614–620.
33. Basu S, Smedman A & Vessby B (2000) Conjugated linoleic acid induces lipid peroxidation in humans. *FEBS Lett* **468**, 33–36.
34. Risérus U, Vessby B, Arner P, *et al.* (2004) Supplementation with *trans*10*cis*12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. *Diabetologia* **47**, 1016–1019.
35. Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, *et al.* (1999) Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARα. *J Lipid Res* **40**, 1426–1433.
36. Moya-Camarena SY, Van den Heuvel JP & Belury MA (1999) Conjugated linoleic acid activates peroxisome proliferator-activated receptor α and β subtypes but does not induce hepatic peroxisome proliferation in Sprague–Dawley rats. *Biochim Biophys Acta* **1436**, 331–342.
37. Yu Y, Correll PH & Vanden Heuvel JP (2002) Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPARγ-dependent mechanism. *Biochim Biophys Acta* **1581**, 89–99.
38. Lemberger T, Desvergne B & Wahli W (1996) Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu Rev Cell Dev Biol* **12**, 335–363.
39. Desvergne B & Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* **20**, 649–688.
40. Chinetti G, Fruchart JC & Staels B (2000) Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res* **49**, 497–505.
41. Duval C, Chinetti G, Trottein F, *et al.* (2002) The role of PPARs in atherosclerosis. *Trends Mol Med* **8**, 422–430.
42. Schoonjans K, Martin G, Staels B, *et al.* (1997) Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr Opin Lipidol* **8**, 159–166.
43. Tan NS, Michalik L, Desvergne B, *et al.* (2005) Multiple expression control mechanisms of peroxisome proliferator-activated receptors and their target genes. *J Steroid Biochem Mol Biol* **93**, 99–105.
44. Qi C, Zhu Y & Reddy JK (2000) Peroxisome proliferator-activated receptors, coactivators, and downstream targets. *Cell Biochem Biophys* **32**, 187–204.
45. Mandard S, Müller M & Kersten S (2004) Peroxisome proliferator receptor α target genes. *Cell Mol Life Sci* **61**, 393–416.
46. Kersten S, Seydoux J, Peters JM, *et al.* (1999) Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *J Clin Invest* **103**, 1489–1498.
47. Straus DS, Pascual G, Li M, *et al.* (2000) 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway. *Proc Natl Acad Sci U S A* **97**, 4844–4849.
48. Wang P, Anderson PO, Chen S, *et al.* (2001) Inhibition of the transcription factors AP-1 and NF-κB in CD4 T cells by peroxisome proliferator-activated receptor γ ligands. *Int Immunopharmacol* **1**, 803–812.
49. Clark RB, Bishop-Bailey D, Estrada-Hernandez T, *et al.* (2000) The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses. *J Immunol* **164**, 1364–1371.
50. Delerive P, Gervois P, Fruchart JC, *et al.* (2000) Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators. *J Biol Chem* **275**, 36703–36707.
51. De Martin R, Hoeth M, Hofer-Warbinek R, *et al.* (2000) The transcription factor NF-κB and the regulation of vascular cell function. *Arterioscler Thromb Vasc Biol* **20**, E83–E88.
52. Barnes PJ & Karin M (1997) Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* **336**, 1066–1071.

British Journal of Nutrition

53. Collins T & Cybulsky MI (2001) NF-κB: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest* **107**, 255–264.
54. Jones WK, Brown M, Wilhide M, *et al.* (2005) NF-κB in cardiovascular disease: diverse and specific effects of a "general" transcription factor? *Cardiovasc Toxicol* **5**, 183–202.
55. Marx N, Duez H, Fruchart JC, *et al.* (2004) Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res* **94**, 1168–1178.
56. Saxena U & Goldberg IJ (1994) Endothelial cells and atherosclerosis: lipoprotein metabolism, matrix interactions, and monocyte recruitment. *Curr Opin Lipidol* **5**, 316–322.
57. Nilsson J (1993) Cytokines and smooth muscle cells in atherosclerosis. *Cardiovasc Res* **27**, 1184–1190.
58. Linton MF & Fazio S (2003) Macrophages, inflammation, and atherosclerosis. *Int J Obes Relat Metab Disord* **27**, Suppl. 3, S35–S40.
59. Toborek M & Kaiser S (1999) Endothelial cell functions. Relationship to atherogenesis. *Basic Res Cardiol* **94**, 295–314.
60. Bombeli T, Mueller M & Haeberli A (1997) Anticoagulant properties of the vascular endothelium. *Thromb Haemost* **77**, 408–423.
61. Cines DB, Pollak ES, Buck CA, *et al.* (1998) Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* **91**, 3527–3561.
62. Schwartz SM (1980) Role of endothelial integrity in atherosclerosis. *Artery* **8**, 305–314.
63. Bevilacqua MP, Nelson RM, Mannori G, *et al.* (1994) Endothelial-leukocyte adhesion molecules in human disease. *Annu Rev Med* **45**, 361–378.
64. O'Brien KD, McDonald TO, Chait A, *et al.* (1996) Neovascular expression of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in human atherosclerosis and their relation to intimal leukocyte content. *Circulation* **93**, 672–682.
65. Barger AC, Beeuwkes R III, Lainey LL, *et al.* (1984) Hypothesis: vasa vasorum and neovascularization of human coronary arteries. *N Engl J Med* **310**, 175–177.
66. Zhang Y, Cliff WJ, Schoefl GI, *et al.* (1993) Immunohistochemical study of intimal microvessels in coronary atherosclerosis. *Am J Pathol* **143**, 164–172.
67. Jackson SM, Parhami F, Xi XP, *et al.* (1999) Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte–endothelial cell interaction. *Arterioscler Thromb Vasc Biol* **19**, 2094–2104.
68. Marx N, Mach F, Sauty A, *et al.* (2000) PPARγ activators inhibit interferon-γ-induced expression of the T cell-active CXC chemokines IP-10, Mig, and I-TAC in human endothelial cells. *J Immunol* **164**, 6503–6508.
69. Sasaki M, Jordan P, Welbourne T, *et al.* (2005) Troglitazone, a PPAR-γ activator prevents endothelial cell adhesion molecule expression and lymphocyte adhesion mediated by TNF-α. *BMC Physiol* **5**, 3.
70. Collins T (1993) Endothelial nuclear factor-κB and the initiation of the atherosclerotic lesion. *Lab Invest* **68**, 499–508.
71. Collins T, Read MA, Neish AS, *et al.* (1995) Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. *FASEB J* **9**, 899–909.
72. Krieglstein CF & Granger DN (2001) Adhesion molecules and their role in vascular disease. *Am J Hypertens* **14**, 44S–54S.
73. Dustin ML, Rothlein R, Bhan AK, *et al.* (1986) Induction by IL 1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J Immunol* **137**, 245–254.
74. Schleser S, Ringseis R & Eder K (2006) Conjugated linoleic acids have no effect on TNFα-induced adhesion molecule expression, U937 monocyte adhesion, and chemokine release in human aortic endothelial cells. *Atherosclerosis* **186**, 337–344.
75. Sneddon AA, McLeod E, Wahle KW, *et al.* (2006) Cytokine-induced monocyte adhesion to endothelial cells involves platelet-activating factor: suppression by conjugated linoleic acid. *Biochim Biophys Acta* **1761**, 793–801.
76. Huang YC, Luedecke LO & Shultz TD (1994) Effect of Cheddar cheese consumption on plasma conjugated linoleic acid concentrations in men. *Nutr Res* **14**, 373–386.
77. Lavillonniere F, Martin JC, Bougnoux P, *et al.* (1998) Analysis of conjugated linoleic acid isomers and content in French cheeses. *J Am Oil Chem Soc* **75**, 343–352.
78. Burdge GC, Lupoli B, Russell JJ, *et al.* (2004) Incorporation of *cis*-9,*trans*-11 or *trans*-10,*cis*-12 conjugated linoleic acid into plasma and cellular lipids in healthy men. *J Lipid Res* **45**, 736–741.
79. Shen J, Halenda SP, Sturek M, *et al.* (2005) Novel mitogenic effect of adenosine on coronary artery smooth muscle cells: role for the A_1 adenosine receptor. *Circ Res* **96**, 982–990.
80. Zacharia LC, Jackson EK, Gillespie DG, *et al.* (2001) Increased 2-methoxyestradiol production in human coronary versus aortic vascular cells. *Hypertension* **37**, 658–662.
81. Ryder JW, Portocarrero CP, Song XM, *et al.* (2001) Isomer-specific antidiabetic properties of conjugated linoleic acid: improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. *Diabetes* **50**, 1149–1157.
82. Evans M, Brown J & McIntosh M (2002) Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism. *J Nutr Biochem* **13**, 508.
83. Nugent AP, Roche HM, Noone EJ, *et al.* (2005) The effects of conjugated linoleic acid supplementation on immune function in healthy volunteers. *Eur J Clin Nutr* **59**, 742–750.
84. Jenko KJ & Vanderhoek JY (2008) Conjugated linoleic acids and CLA-containing phospholipids inhibit NO formation in aortic endothelial cells. *Lipids* **43**, 335–342.
85. Coen P, Cummins P, Birney Y, *et al.* (2004) Modulation of nitric oxide and 6-keto-prostaglandin F(1α) production in bovine aortic endothelial cells by conjugated linoleic acid. *Endothelium* **11**, 211–220.
86. Eder K, Schleser S, Becker K, *et al.* (2003) Conjugated linoleic acids lower the release of eicosanoids and nitric oxide from human aortic endothelial cells. *J Nutr* **133**, 4083–4089.
87. Dancu MB, Berardi DE, Vanden Heuvel JP, *et al.* (2007) Atherogenic endothelial cell eNOS and ET-1 responses to asynchronous hemodynamics are mitigated by conjugated linoleic acid. *Ann Biomed Eng* **35**, 1111–1119.
88. Torres-Duarte AP & Vanderhoek JY (2003) Conjugated linoleic acid exhibits stimulatory and inhibitory effects on prostanoid production in human endothelial cells and platelets. *Biochim Biophys Acta* **1640**, 69–76.
89. Urquhart P, Parkin SM, Rogers JS, *et al.* (2002) The effect of conjugated linoleic acid on arachidonic acid metabolism and eicosanoid production in human saphenous vein endothelial cells. *Biochim Biophys Acta* **1580**, 150–160.
90. Pearson JD (2000) Normal endothelial cell function. *Lupus* **9**, 183–188.
91. Jansson PA (2007) Endothelial dysfunction in insulin resistance and type 2 diabetes. *J Intern Med* **262**, 173–183.
92. Kalogeropoulou K, Mortzos G, Migdalis I, *et al.* (2002) Carotid atherosclerosis in type 2 diabetes mellitus: potential role of endothelin-1, lipoperoxides, and prostacyclin. *Angiology* **53**, 279–285.
93. Delerive P, Martin-Nizard F, Chinetti G, *et al.* (1999) Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. *Circ Res* **85**, 394–402.

94. Satoh H, Tsukamoto K, Hashimoto Y, *et al.* (1999) Thiazolidinediones suppress endothelin-1 secretion from bovine vascular endothelial cells: a new possible role of PPARγ on vascular endothelial function. *Biochem Biophys Res Commun* **254**, 757–763.
95. Degner SC, Kemp MQ, Bowden GT, *et al.* (2006) Conjugated linoleic acid attenuates cyclooxygenase-2 transcriptional activity via an anti-AP-1 mechanism in MCF-7 breast cancer cells. *J Nutr* **136**, 421–427.
96. Sheu JN, Lin TH, Lii CK, *et al.* (2006) Contribution of conjugated linoleic acid to the suppression of inducible nitric oxide synthase expression and transcription factor activation in stimulated mouse mesangial cells. *Food Chem Toxicol* **44**, 409–416.
97. Lu D, Maulik N, Moraru II, *et al.* (1993) Molecular adaptation of vascular endothelial cells to oxidative stress. *Am J Physiol* **264**, C715–C722.
98. Cross CE, Halliwell B, Borish ET, *et al.* (1987) Oxygen radicals and human disease. *Ann Intern Med* **107**, 526–545.
99. Aviram M (2000) Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. *Free Radic Res* **33**, S85–S97.
100. Sneddon AA, Wu HC, Farquharson A, *et al.* (2003) Regulation of selenoprotein GPx4 expression and activity in human endothelial cells by fatty acids, cytokines and antioxidants. *Atherosclerosis* **171**, 57–65.
101. Hao H, Gabbiani G & Bochaton-Piallat ML (2003) Arterial smooth muscle cell heterogeneity: implications for atherosclerosis and restenosis development. *Arterioscler Thromb Vasc Biol* **23**, 1510–1520.
102. Schwartz SM, Campbell GR & Campbell JH (1986) Replication of smooth muscle cells in vascular disease. *Circ Res* **58**, 427–444.
103. Yutani C, Fujita H, Takaichi S, *et al.* (1993) The role of vascular smooth muscle cell phenotypic modulation at the aortic branch in atherogenesis. *Front Med Biol Eng* **5**, 143–146.
104. Stary HC, Chandler B, Dinsmore RE, *et al.* (1995) A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. *Circulation* **92**, 1355–1374.
105. Katsuda S & Kaji T (2003) Atherosclerosis and extracellular matrix. *J Atheroscler Thromb* **10**, 267–274.
106. Burke JM & Ross R (1979) Synthesis of connective tissue macromolecules by smooth muscle. *Int Rev Connect Tissue Res* **8**, 119–157.
107. Jimenez R, Belcher E, Sriskandan S, *et al.* (2005) Role of Toll-like receptors 2 and 4 in the induction of cyclooxygenase-2 in vascular smooth muscle. *Proc Natl Acad Sci U S A* **102**, 4637–4642.
108. Corsini A, Verri D, Raiteri M, *et al.* (1995) Effects of 26-aminocholesterol, 27-hydroxycholesterol, and 25-hydroxycholesterol on proliferation and cholesterol homeostasis in arterial myocytes. *Arterioscler Thromb Vasc Biol* **15**, 420–428.
109. Ringseis R, Müller A, Herter C, *et al.* (2006) CLA isomers inhibit TNFα-induced eicosanoid release from human vascular smooth muscle cells via a PPARγ ligand-like action. *Biochim Biophys Acta* **1760**, 290–300.
110. Ringseis R, Gahler S, Herter C, *et al.* (2006) Conjugated linoleic acids exert similar actions on prostanoid release from aortic and coronary artery smooth muscle cells. *Int J Vit Nutr Res* **76**, 281–289.
111. Cheng WL, Lii CK, Chen HW, *et al.* (2004) Contribution of conjugated linoleic acid to the suppression of inflammatory responses through the regulation of the NF-κB pathway. *J Agric Food Chem* **52**, 71–78.
112. Eder K, Slomma N & Becker K (2002) *Trans*-10,*cis*-12 conjugated linoleic acid suppresses the desaturation of linoleic and α-linolenic acids in HepG2 cells. *J Nutr* **132**, 1115–1121.
113. Stachowska E, Dziedziejko V, Safranow K, *et al.* (2007) Inhibition of phospholipase A2 activity by conjugated linoleic acids in human macrophages. *Eur J Nutr* **46**, 28–33.
114. Han WK, Sapirstein A, Hung CC, *et al.* (2003) Cross-talk between cytosolic phospholipase A2α (cPLA2α) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2α activity that is responsible for arachidonic acid release. *J Biol Chem* **278**, 24153–24163.
115. Nugteren DH & Christ-Hazelhof E (1987) Naturally occurring conjugated octadecatrienoic acids are strong inhibitors of prostaglandin biosynthesis. *Prostaglandins* **33**, 403–417.
116. Bulgarella JA, Patton D & Bull AW (2001) Modulation of prostaglandin H synthase activity by conjugated linoleic acid (CLA) and specific CLA isomers. *Lipids* **36**, 407–412.
117. Banni S, Petroni A, Blasevich M, *et al.* (2004) Detection of conjugated C16 PUFAs in rat tissues as possible partial β-oxidation products of naturally occurring conjugated linoleic acid and its metabolites. *Biochim Biophys Acta* **1682**, 120–127.
118. Sébédio JL, Angioni E, Chardigny JM, *et al.* (2001) The effect of conjugated linoleic acid isomers on fatty acid profiles of liver and adipose tissues and their conversion to isomers of 16:2 and 18:3 conjugated fatty acids in rats. *Lipids* **36**, 575–582.
119. Müller A, Ringseis R, Düsterloh K, *et al.* (2005) Detection of conjugated dienoic fatty acids in human vascular smooth muscle cells treated with conjugated linoleic acid. *Biochim Biophys Acta* **1737**, 145–151.
120. Ringseis R, Müller A, Düsterloh K, *et al.* (2006) Formation of conjugated linoleic acid metabolites in human vascular endothelial cells. *Biochim Biophys Acta* **1761**, 377–383.
121. Müller A, Mickel M, Geyer R, *et al.* (2006) Identification of conjugated linoleic acid elongation and β-oxidation products by coupled silver-ion HPLC APPI-MS. *J Chromatogr B* **837**, 147–152.
122. Garcia MC, Sprecher H & Rosenthal MD (1990) Chain elongation of polyunsaturated fatty acids by vascular endothelial cells: studies with arachidonate analogues. *Lipids* **25**, 211–215.
123. Morisaki N, Kanzaki T, Fujiyama Y, *et al.* (1985) Metabolism of *n*-3 polyunsaturated fatty acids and modification of phospholipids in cultured rabbit aortic smooth muscle cells. *J Lipid Res* **26**, 930–939.
124. Morita I, Schindler M, Regier MK, *et al.* (1995) Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J Biol Chem* **270**, 10902–10908.
125. Mukherjee E & Ghosh D (1989) Subcellular localization of cyclooxygenase-prostaglandin E_2 synthetase complex in goat vesicular gland by catalytic activity analysis. *Prostaglandins* **38**, 557–563.
126. Brock TG, McNish RW & Peters-Golden M (1999) Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E_2. *J Biol Chem* **274**, 11660–11666.
127. Ikeda U, Shimpo M, Murakami Y, *et al.* (2000) Peroxisome proliferator-activated receptor-γ ligands inhibit nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* **35**, 1232–1236.
128. Marx N, Schönbeck U, Lazar MA, *et al.* (1998) Peroxisome proliferator-activated receptor γ activators inhibit gene expression and migration in human vascular smooth muscle cells. *Circ Res* **83**, 1097–1103.
129. Park KG, Lee KM, Chang YC, *et al.* (2006) The ascochlorin derivative, AS-6, inhibits TNF-α-induced adhesion molecule and chemokine expression in rat vascular smooth muscle cells. *Life Sci* **80**, 120–126.

British Journal of Nutrition

130. Takata Y, Kitami Y, Yang ZH, *et al.* (2002) Vascular inflammation is negatively autoregulated by interaction between CCAAT/enhancer-binding protein-δ and peroxisome proliferator-activated receptor-γ. *Circ Res* **91**, 427–433.
131. Goua M, Mulgrew S, Frank J, *et al.* (2008) Regulation of adhesion molecule expression in human endothelial and smooth muscle cells by omega-3 fatty acids and conjugated linoleic acids: involvement of the transcription factor NF-κB? *Prostaglandins Leukot Essent Fatty Acids* **78**, 33–43.
132. Price DT & Lascalzo J (1999) Cellular adhesion molecules and atherogenesis. *Am J Med* **107**, 85–97.
133. Davies MJ, Gordon JL, Gearing AJ, *et al.* (1993) The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol* **171**, 223–229.
134. Ringseis R, Gahler S & Eder K (2008) Conjugated linoleic acid isomers inhibit platelet-derived growth factor-induced NF-κB transactivation and collagen formation in human vascular smooth muscle cells. *Eur J Nutr* **47**, 59–67.
135. Jia Y & Turek J (2005) Altered NF-κB gene expression and collagen formation induced by polyunsaturated fatty acids. *J Nutr Biochem* **16**, 500–506.
136. Jimi S, Saku K, Uesugi N, *et al.* (1995) Oxidized low density lipoprotein stimulates collagen production in cultured arterial smooth muscle cells. *Atherosclerosis* **116**, 15–26.
137. Büttner C, Skupin A & Rieber EP (2004) Transcriptional activation of the type I collagen genes COL1A1 and COL1A2 in fibroblasts by interleukin-4: analysis of the functional collagen promoter sequences. *J Cell Physiol* **198**, 248–258.
138. Nieto N, Friedman SL, Greenwel P, *et al.* (1999) CYP2E1-mediated oxidative stress induces collagen type I expression in rat hepatic stellate cells. *Hepatology* **30**, 987–996.
139. Nieto N, Greenwel P, Friedman SL, *et al.* (2000) Ethanol and arachidonic acid increase a 2(I) collagen expression in rat hepatic stellate cells overexpressing cytochrome P450 2E1. Role of H_2O_2 and cyclooxygenase-2. *J Biol Chem* **275**, 20136–20145.
140. Bowie A & O'Neill LA (2000) Oxidative stress and nuclear factor-κB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem Pharmacol* **59**, 13–23.
141. Chen A, Zhang L, Xu J, *et al.* (2002) The antioxidant (−)-epigallocatechin-3-gallate inhibits activated hepatic stellate cell growth and suppresses acetaldehyde-induced gene expression. *Biochem J* **368**, 695–704.
142. Fu M, Zhang J, Zhu X, *et al.* (2001) Peroxisome proliferator-activated receptor c inhibits transforming growth factor β-induced connective tissue growth factor expression in human aortic smooth muscle cells by interfering with Smad3. *J Biol Chem* **276**, 45888–45894.
143. Cernuda-Morollón E, Pineda-Molina E, Cañada FJ, *et al.* (2001) 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibition of NF-κB-DNA binding through covalent modification of the p50 subunit. *J Biol Chem* **276**, 35530–35536.
144. Levick SP, Loch DC, Taylor SM, *et al.* (2007) Arachidonic acid metabolism as a potential mediator of cardiac fibrosis associated with inflammation. *J Immunol* **178**, 641–646.
145. LaPointe MC, Mendez M, Leung A, *et al.* (2004) Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. *Am J Physiol* **286**, H1416–H1424.
146. Muz MH, Deveci F, Bulut Y, *et al.* (2006) The effects of low dose leukotriene receptor antagonist therapy on airway remodeling and cysteinyl leukotriene expression in a mouse asthma model. *Exp Mol Med* **38**, 109–118.
147. Suganami T, Mori K, Tanaka I, *et al.* (2003) Role of prostaglandin E receptor EP1 subtype in the development of renal injury in genetically hypertensive rats. *Hypertension* **42**, 1183–1190.
148. Gizard F, Amant C, Barbier O, *et al.* (2005) PPARα inhibits vascular smooth muscle cell proliferation underlying intimal hyperplasia by inducing the tumor suppressor p16INK4a. *J Clin Invest* **115**, 3228–3238.
149. Miwa Y, Sasaguri T, Inoue H, *et al.* (2000) 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 induces G_1 arrest and differentiation marker expression in vascular smooth muscle cells. *Mol Pharmacol* **58**, 837–844.
150. Gouni-Berthold I, Berthold HK, Weber AA, *et al.* (2001) Troglitazone and rosiglitazone inhibit the low density lipoprotein-induced vascular smooth muscle cell growth. *Exp Clin Endocrinol Diabetes* **109**, 203–209.
151. Kim EJ, Shin HK, Cho JS, *et al.* (2006) *Trans*-10,*cis*-12 conjugated linoleic acid inhibits the G_1-S cell cycle progression in DU145 human prostate carcinoma cells. *J Med Food* **9**, 293–299.
152. Kemp MQ, Jeffy BD & Romagnolo DF (2003) Conjugated linoleic acid inhibits cell proliferation through a p53-dependent mechanism: effects on the expression of G_1-restriction points in breast and colon cancer cells. *J Nutr* **133**, 3670–3677.
153. Cho HJ, Kim EJ, Lim SS, *et al.* (2006) *Trans*-10,*cis*-12, not *cis*-9,*trans*-11, conjugated linoleic acid inhibits G_1-S progression in HT-29 human colon cancer cells. *J Nutr* **136**, 893–898.
154. Lim DY, Tyner AL, Park JB, *et al.* (2005) Inhibition of colon cancer cell proliferation by the dietary compound conjugated linoleic acid is mediated by the CDK inhibitor p21CIP1/WAF1. *J Cell Physiol* **205**, 107–113.
155. Moon EJ, Lee YM & Kim KW (2003) Anti-angiogenic activity of conjugated linoleic acid on basic fibroblast growth factor-induced angiogenesis. *Oncol Rep* **10**, 617–621.
156. Lai KL, Torres-Duarte AP & Vanderhoek JY (2005) 9-*Trans*, 11-*trans*-CLA: antiproliferative and proapoptotic effects on bovine endothelial cells. *Lipids* **40**, 1107–1116.
157. Ulrich-Merzenich G, Metzner C, Schiermeyer B, *et al.* (2002) Vitamin C and vitamin E antagonistically modulate human vascular endothelial and smooth muscle DNA synthesis and proliferation. *Eur J Nutr* **41**, 27–34.
158. Martin S, Favot L, Matz R, *et al.* (2003) Delphinidin inhibits endothelial cell proliferation and cell cycle progression through a transient activation of ERK-1/-2. *Biochem Pharmacol* **65**, 669–675.
159. Ylä-Herttuala S, Lipton BA, Rosenfeld ME, *et al.* (1991) Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci U S A* **88**, 5252–5256.
160. Ylä-Herttuala S (1991) Macrophages and oxidized low density lipoproteins in the pathogenesis of atherosclerosis. *Ann Med* **23**, 561–567.
161. de Villiers WJ & Smart EJ (1999) Macrophage scavenger receptors and foam cell formation. *J Leukoc Biol* **66**, 740–746.
162. Stachowska E, Dziedziejko V, Safranow K, *et al.* (2007) Effect of conjugated linoleic acids on the activity and mRNA expression of 5- and 15-lipoxygenases in human macrophages. *J Agric Food Chem* **55**, 5335–5342.
163. Stachowska E, Baskiewicz-Masiuk M, Dziedziejko V, *et al.* (2007) Conjugated linoleic acids can change phagocytosis of human monocytes/macrophages by reduction in Cox-2 expression. *Lipids* **42**, 707–716.
164. Iwakiri Y, Sampson DA & Allen KG (2002) Suppression of cyclooxygenase-2 and inducible nitric oxide synthase expression by conjugated linoleic acid in murine macrophages. *Prostaglandins Leukot Essent Fatty Acids* **67**, 435–443.
165. Yang M & Cook ME (2003) Dietary conjugated linoleic acid decreased cachexia, macrophage tumor necrosis factor-α production, and modifies splenocyte cytokines production. *Exp Biol Med (Maywood)* **228**, 51–58.

British Journal of Nutrition

166. Rahman MM, Bhattacharya A & Fernandes G (2006) Conjugated linoleic acid inhibits osteoclast differentiation of RAW264.7 cells by modulating RANKL signalling. *J Lipid Res* **47**, 1739–1748.
167. Song DH, Kang JH, Lee GS, *et al.* (2007) Upregulation of tumor necrosis factor-α expression by *trans*10-*cis*12 conjugated linoleic acid enhances phagocytosis of RAW macrophages via a peroxisome proliferator-activated receptor γ-dependent pathway. *Cytokine* **37**, 227–235.
168. Kang JH, Lee GS, Jeung EB, *et al.* (2007) *Trans*-10,*cis*-12-conjugated linoleic acid increases phagocytosis of porcine peripheral blood polymorphonuclear cells *in vitro*. *Br J Nutr* **97**, 117–125.
169. Meier CA, Chicheportiche R, Juge-Aubry CE, *et al.* (2002) Regulation of the interleukin-1 receptor antagonist in THP-1 cells by ligands of the peroxisome proliferator-activated receptor γ. *Cytokine* **18**, 320–328.
170. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, *et al.* (2007) Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. *Nature* **447**, 1116–1120.
171. Bouhlel MA, Derudas B, Rigamonti E, *et al.* (2007) PPARγ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab* **6**, 137–143.
172. Ricote M, Li AC, Willson TM, *et al.* (1998) The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* **391**, 79–82.
173. Jiang C, Ting AT & Seed B (1998) PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* **391**, 82–86.
174. Lampen A, Leifheit M, Voss J, *et al.* (2005) Molecular and cellular effects of *cis*-9, *trans*-11-conjugated linoleic acid in enterocytes: effects on proliferation, differentiation, and gene expression. *Biochim Biophys Acta* **1735**, 30–40.
175. Welch JS, Ricote M, Akiyama TE, *et al.* (2003) PPARγ and PPARδ negatively regulate specific subsets of lipopolysaccharide and IFN-γ target genes in macrophages. *Proc Natl Acad Sci U S A* **100**, 6712–6717.
176. Neve BP, Corseaux D, Chinetti G, *et al.* (2001) PPARα agonists inhibit tissue factor expression in human monocytes and macrophages. *Circulation* **103**, 207–212.
177. Lee CH, Chawla A, Urbiztondo N, *et al.* (2003) Transcriptional repression of atherogenic inflammation: modulation by PPARδ. *Science* **302**, 453–457.
178. Ringseis R, Wen G, Saal D, *et al.* (2008) Conjugated linoleic acid isomers reduce cholesterol accumulation in acetylated LDL-induced mouse RAW264.7 macrophage-derived foam cells. *Lipids* **43**, 913–923.
179. Weldon S, Mitchell S, Kelleher D, *et al.* (2004) Conjugated linoleic acid and atherosclerosis: no effect on molecular markers of cholesterol homeostasis in THP-1 macrophages. *Atherosclerosis* **174**, 261–273.
180. Chinetti G, Lestavel S, Bocher V, *et al.* (2001) PPAR-α and PPAR-γ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* **7**, 53–58.
181. Chinetti G, Lestavel S, Fruchart JC, *et al.* (2003) Peroxisome proliferator-activated receptor α reduces cholesterol esterification in macrophages. *Circ Res* **92**, 212–217.
182. Chawla A, Boisvert WA, Lee CH, *et al.* (2001) PPARγ–LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* **7**, 161–171.
183. Moore KJ, Rosen ED, Fitzgerald ML, *et al.* (2001) The role of PPAR-γ in macrophage differentiation and cholesterol uptake. *Nat Med* **7**, 41–47.
184. Chinetti-Gbaguidi G, Rigamonti E, Helin L, *et al.* (2005) Peroxisome proliferator-activated receptor α controls cellular cholesterol trafficking in macrophages. *J Lipid Res* **46**, 2717–2725.
185. Akiyama TE, Sakai S, Lambert G, *et al.* (2002) Conditional disruption of the peroxisome proliferator-activated receptor γ gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. *Mol Cell Biol* **22**, 2607–2619.
186. Bodzioch M, Orsó E, Klucken J, *et al.* (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet* **22**, 347–351.
187. Brooks-Wilson A, Marcil M, Clee SM, *et al.* (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet* **22**, 336–345.
188. Rust S, Rosier M, Funke H, *et al.* (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter. *Nat Genet* **22**, 352–355.
189. Ecker J, Langmann T, Moehle C, *et al.* (2007) Isomer specific effects of conjugated linoleic acid on macrophage ABCG1 transcription by a SREBP-1c dependent mechanism. *Biochem Biophys Res Commun* **352**, 805–811.
190. Carstea ED, Morris JA, Coleman KG, *et al.* (1997) Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science* **277**, 228–231.
191. Strauss JF III, Liu P, Christenson LK, *et al.* (2002) Sterols and intracellular vesicular trafficking: lessons from the study of NPC1. *Steroids* **67**, 947–951.
192. Hirakata M, Tozawa R, Imura Y, *et al.* (2004) Comparison of the effects of pioglitazone and rosiglitazone on macrophage foam cell formation. *Biochem Biophys Res Commun* **323**, 782–788.
193. Valeille K, Ferezou J, Parquet M, *et al.* (2006) The natural concentration of the conjugated linoleic acid, *cis*-9, *trans*-11, in milk fat has antiatherogenic effects in hyperlipidemic hamsters. *J Nutr* **136**, 1305–1310.
194. Lendon CL, Davies MJ, Born GV, *et al.* (1991) Atherosclerotic plaque caps are locally weakened when macrophages density is increased. *Atherosclerosis* **87**, 87–90.
195. Shah PK, Falk E, Badimon JJ, *et al.* (1995) Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques: potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* **92**, 1565–1569.
196. Gibbs DF, Warner RL, Weiss SJ, *et al.* (1999) Characterization of matrix metalloproteinases produced by rat alveolar macrophages. *Am J Respir Cell Mol Biol* **20**, 1136–1144.
197. Galis ZS, Sukhova GK, Kranzhofer R, *et al.* (1995) Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. *Proc Natl Acad Sci U S A* **92**, 402–406.
198. Shah PK (2003) Mechanisms of plaque vulnerability and rupture. *J Am Coll Cardiol* **41**, 15S–22S.
199. Aikawa M & Libby P (2004) The vulnerable atherosclerotic plaque: pathogenesis and therapeutic approach. *Cardiovasc Pathol* **13**, 125–138.
200. Virmani R, Kolodgie FD, Burke AP, *et al.* (2005) Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* **25**, 2054–2061.
201. Kai H, Ikeda H, Yasukawa H, *et al.* (1998) Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* **32**, 368–372.
202. Zeng B, Prasan A, Fung KC, *et al.* (2005) Elevated circulating levels of matrix metalloproteinase-9 and -2 in patients with symptomatic coronary artery disease. *Intern Med J* **35**, 331–335.
203. Ringseis R, Schulz N, Saal D, *et al.* (2008) Troglitazone but not conjugated linoleic acid reduces gene expression and

activity of matrix-metalloproteinases-2 and -9 in PMA-differentiated THP-1 macrophages. *J Nutr Biochem* **19**, 594–603.
204. Zaltsman AB, George SJ & Newby AC (1999) Increased secretion of tissue inhibitors of metalloproteinases 1 and 2 from the aortas of cholesterol fed rabbits partially counterbalances increased metalloproteinase activity. *Arterioscler Thromb Vasc Biol* **19**, 1700–1707.
205. Albin RJ, Senior RM, Welgus HG, *et al.* (1987) Human alveolar macrophages secrete an inhibitor of metalloproteinase elastase. *Am Rev Respir Dis* **135**, 1281–1285.
206. Lee KJ, Kim HA, Kim PH, *et al.* (2004) Ox-LDL suppresses PMA-induced MMP-9 expression and activity through CD36-mediated activation of PPAR-γ. *Exp Mol Med* **36**, 534–544.
207. Woo JH, Park JW, Lee SH, *et al.* (2003) Dykellic acid inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting nuclear factor κB transcriptional activity. *Cancer Res* **63**, 3430–3434.
208. Han YP, Tuan TL, Wu H, *et al.* (2001) TNF-α stimulates activation of pro-MMP2 in human skin through NF-κB mediated induction of MT1-MMP. *J Cell Sci* **114**, 131–139.
209. Logan SK, Garabedian MJ, Campbell CE, *et al.* (1996) Synergistic transcriptional activation of the tissue inhibitor of metalloproteinases-1 promoter via functional interaction of AP-1 and Ets-1 transcription factors. *J Biol Chem* **271**, 774–782.
210. Hah N & Lee ST (2003) An absolute role of the PKC-dependent NF-κB activation for induction of MMP-9 in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* **305**, 428–433.
211. Lehmann JM, Moore LB, Smith-Oliver TA, *et al.* (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). *J Biol Chem* **270**, 12953–12956.
212. Kritchevsky D, Tepper SA, Wright S, *et al.* (2002) Influence of graded levels of conjugated linoleic acid (CLA) on experimental atherosclerosis in rabbits. *Nutr Res* **22**, 1275–1279.
213. Kritchevsky D, Tepper SA, Wright S, *et al.* (2004) Conjugated linoleic acid isomer effects in atherosclerosis: growth and regression of lesions. *Lipids* **39**, 611–616.
214. Nicolosi RJ, Rogers EJ, Kritchevsky D, *et al.* (1997) Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. *Artery* **22**, 266–277.
215. Mitchell PL, Langille MA, Currie DL, *et al.* (2005) Effect of conjugated linoleic acid isomers on lipoproteins and atherosclerosis in the Syrian Golden hamster. *Biochim Biophys Acta* **1734**, 269–276.
216. Arbonés-Mainar JM, Navarro MA, Guzmán MA, *et al.* (2006) Selective effect of conjugated linoleic acid isomers on atherosclerotic lesion development in apolipoprotein E knockout mice. *Atherosclerosis* **189**, 318–327.
217. Tholstrup T, Raff M, Straarup EM, *et al.* (2008) An oil mixture with *trans*-10, *cis*-12 conjugated linoleic acid increases markers of inflammation and *in vivo* lipid peroxidation compared with *cis*-9, *trans*-11 conjugated linoleic acid in postmenopausal women. *J Nutr* **138**, 1445–1451.
218. Poirier H, Shapiro JS, Kim RJ, *et al.* (2006) Nutritional supplementation with *trans*-10, *cis*-12-conjugated linoleic acid induces inflammation of white adipose tissue. *Diabetes* **55**, 1634–1641.
219. Chung S, Brown JM, Provo JN, *et al.* (2005) Conjugated linoleic acid promotes human adipocyte insulin resistance through NF-κB-dependent cytokine production. *J Biol Chem* **280**, 38445–38456.
220. Risérus U, Arner P, Brismar K, *et al.* (2002) Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* **25**, 1516–1521.

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