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REVIEW-THEMED ISSUE

Atheroprotective effects of conjugated linoleic acid

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Atherosclerosis, the underlying cause of heart attack and strokes, is a progressive dyslipidaemic and inflammatory disease where monocyte-derived macrophage cells play a pivotal role. Although most of the mechanisms that contribute to the progression of atherosclerosis have been identified, there is limited information on those governing regression. Conjugated linoleic acid (CLA) is a generic term denoting a group of naturally occurring isomers of linoleic acid (18:2, n6) that differ in the position or geometry (i.e. *cis* or *trans*) of their double bonds. The most predominant isomers in ruminant fats are *cis*-9, *trans*-11 CLA (c9,t11-CLA), which accounts for more than 80% of CLA isomers in dairy products and *trans*-10, *cis*-12 CLA (t10,c12-CLA). Dietary administration of a blend of the two most abundant isomers of CLA has been shown to inhibit the progression and induce the regression of pre-established atherosclerosis. Studies investigating the mechanisms involved in CLA-induced atheroprotective effects are continually emerging. The purpose of this review is to discuss comprehensively the effects of CLA on monocyte/macrophage function in atherosclerosis and to identify possible mechanisms through which CLA mediates its atheroprotective effects.

Atherosclerosis

Atherosclerosis is a complex inflammatory disease that is characterized by the progressive formation of lipid laden fibrous plaques within the arterial wall [1]. This chronic disease arises from a maladaptive inflammatory response, an impaired resolution process and a defective lipid metabolism [2]. Progressive damage to the vessel wall culminates in arterial occlusion resulting in stenosis or lesion rupture triggering thrombosis. Atherosclerosis is the underlying cause of ischaemic events and often the first clinical manifestation of atherosclerosis is myocardial infarction or stroke [3]. There are multiple risk factors associated with the development of atherosclerosis. The non-modifiable risk factors include ageing [4], gender [5] and family history [6]. The modifiable risk factors include elevated concentrations of low density lipoprotein (LDL) cholesterol [7], low concentrations of high density lipoprotein (HDL) cholesterol [8], elevated blood pressure [9], diabetes [10], metabolic syndrome [11], obesity [12], physical inactivity [13], cigarette smoking [14, 15] and a diet high in saturated fat, *trans* fat [16] and cholesterol.

The development of the atherosclerotic lesion is initiated by endothelial dysfunction at arterial branch points or locations of altered blood flow [2]. These endothelial alterations facilitate the passage and retention of macromolecules such as LDL molecules within the intima layer [17]. Subsequent oxidation of LDL within the subendothelium by reactive oxygen species (ROS) triggers an inflammatory response [18] characterized by the recruitment of inflammatory cells to the site of endothelial damage. The majority of leukocytes within the developing atherosclerotic lesion are monocytes and macrophages [19]. Oxidized LDL (oxLDL), direct arterial injury, cytokines and growth factors stimulate the secretion of chemokines such as monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-8 from endothelial cells, smooth muscle cells and leukocytes [20]. Both MCP-1 and oxLDL recruit monocytes to the vessel wall [18, 21].

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Monocyte–endothelial interaction is necessary for the extravasation of circulating monocytes into the tissue [22]. This interaction is mediated by chemokines which facilitate the recruitment, tethering and rolling processes that occur at the vessel wall. Monocyte adhesion to the vessel wall is facilitated by key adhesion ligands on monocytes, β_1-integrin cluster of differentiation (CD)49d/CD29 (VLA-4) and β_2-integrin CD11b/CD18 (Mac-1). These ligands bind to vascular cell adhesion molecule 1 (VCAM-1) and intracellular cell adhesion molecule 1 (ICAM-1) located on endothelial cells [23, 24]. This receptor–ligand interaction facilitates the transendothelial migration of monocytes into the vessel intima. Macrophage colony stimulating factor (M-CSF) induces the differentiation of monocytes to macrophages [25]. Macrophages respond to extracellular and intracellular signals such as the tissue microenvironment [26] and lipid derivatives within the intracellular environment [27]. These factors determine macrophage phenotypic polarization resulting in high levels of heterogeneity and plasticity among macrophage subpopulations [28].

The predominant macrophage phenotypes are characterized as MΦ1 'classically' activated and MΦ2 'alternatively' activated. Macrophages exhibit plasticity as they can interchange between MΦ1 and MΦ2 based on intercellular and extracellular stimuli [29]. MΦ1 polarization is induced by T helper (T_H) 1 cytokines including interferon-gamma (IFN-γ), IL-1β, and the gram negative bacterial toxin, lipopolysaccharide (LPS). This MΦ1 subset has a pro-inflammatory phenotype secreting pro-inflammatory cytokines and mediators such as tumour necrosis factor-alpha (TNF-α), IL-6 and IL-12. MΦ2 polarization is induced by certain lipid products and by several T_H2 cytokines including IL-4, IL-13 and IL-10 [30, 31]. The MΦ2 subset has an anti-inflammatory or pro-resolving profile as they produce IL-10, IL-1 receptor antagonist (RA) and transforming growth factor-beta (TGF-β) [31]. In addition, they increase efferocytosis of debris and promote a pro-resolving environment.

Through receptor-mediated endocytosis macrophages take up oxLDL via CD36 and scavenger receptor (SR)-A1. These macrophages have a cholesterol transport system in which processed cholesterol is effluxed via ATP-binding cassette transporter A1 (ABCA-1), ABCG-1 and SR-BI proteins to acceptor molecules to prevent the internal accumulation of excess LDL and free cholesterol [32]. Apolipoprotein (apo)-A1 and HDL, acceptor molecules, shuttle the cholesterol to the liver as part of a process known as reverse cholesterol transport (RCT) [33]. Disruption to this transport system occurs when cholesterol influx exceeds efflux or when acetyl-coA cholesterol acetyltransferase (ACAT)-1-mediated esterification is impaired [34]. This can be due to overloading of lysosomal capacity to transport free cholesterol, a reduction in ABCA-1 and SR-BI, the plasma membrane proteins that mediate efflux or through lack of cholesterol acceptor molecules within the intima [32, 35]. Macrophage foam cells, laden with cholestryl esters, are a predominant feature of early atherosclerotic lesions [36]. Excessive oxLDL accumulation results in foam cell formation and can activate cellular apoptosis or necrosis [37]. Smooth muscle cells and macrophages often undergo apoptosis due to the impaired removal of apoptotic bodies and they can accumulate within the lesion. These apoptotic cells can inhibit maintenance or repair of the extracellular membrane (ECM) thus further destabilizing the plaque [38]. Many plaques consist of apoptotic cells and necrotic cells, along with calcified and fibrotic elements which all contribute to atherosclerotic lesion formation [39]. Park *et al.* demonstrated that oxLDL-CD36 interaction inhibits the egress of murine and human macrophages out of atherosclerotic lesions [40, 41]. This receptor–ligand binding mediates pro-inflammatory effects [42]. Macrophage survival, accumulation and dysfunction impair the resolution process and drive atherosclerotic lesion development.

Most of the current therapies for the treatment of atherosclerosis target one or more risk factors of the disease. These include reduction of circulating LDL cholesterol using statins, anti-hypertensives, anti-coagulants and oral hypoglycaemic agents. However, there are no therapies available which target the inflammatory component of atherosclerosis and importantly there are none available which are designed to mediate regression or promote pro-resolving effects on pre-established disease. The implications of this are important as most patients present with established disease. There is evidence in animal models which suggests that induction of lesion regression may be a viable therapeutic strategy [43]. Reis *et al.* demonstrated remodelling and regression of advanced atherosclerotic lesions in murine models. Administration of a high cholesterol Western diet to apoE knockout ($^{-/-}$) mice, induced hypercholesterolaemia and the formation of advanced atherosclerotic lesions. These lesions were then transplanted to wild type (WT) (normocholestrolaemia) mice and to apoE$^{-/-}$ mice fed on a normal cholesterol diet. Whilst lesions continued to develop in the apoE$^{-/-}$ mice, in the WT mice with a normalized plasma lipoprotein profile, there was a reduction in the lesion size, macrophage content, VCAM-1 expression and foam cell numbers in the intimal and medial layers [44]. This provides evidence that atherosclerotic lesions are dynamic and can undergo remodelling and regression.

Conjugated linoleic acid

CLA is a generic term denoting a group of naturally occurring isomers of linoleic acid (18:2, n6) that differ in the position or geometry (i.e. *cis* or *trans*) of their double bonds [45]. CLA isomers are naturally present in the lipid fraction of ruminant derived products including milk, dairy and beef products [46, 47]. The major source of CLA in the human diet is through the consumption of ruminant meats such as beef and lamb [48] and from high fat dairy products such as whole milk (3% fat), full fat cultured milk, mainly sour milk (3% fat), cheese, mainly hard cheese (28% fat), cream (40% fat), full fat sour cream (34% fat), reduced fat sour cream (17% fat) and butter (80% fat) [49]. Daily consumption of low fat dairy products included low fat milk (0.5% fat), medium fat milk (1.5% fat) and low fat cultured milk (0.5% fat) [49]. Grass fed cattle produce the highest levels of CLA. Cattle fed a diet rich in polyunsaturated fatty acids have increased concentrations of CLA in their milk [50, 51]. There are also dietary CLA supplements that are commercially available. On average humans consume between 15–430 mg of CLA day^{-1} [52]. However in animal studies, CLA effects are only observed at a dose that is approximately 10 times higher [52]. Different

CLA isomers are synthesized through a variety of mechanisms by bacteria and digesta present in the rumen [53]. There are 28 known CLA isomers with c9,t11-CLA, which accounts for 80% of CLA intake in the diet and t10,c12-CLA being the two most abundant. The biological activities of CLA have received considerable attention over the past number of years due to their documented anti-cancer, anti-atherogenic and anti-diabetic effects. However, individual isomers can have divergent effects and their effects are not predictable when combined.

Benefits of CLA on human health

CLA isomers have been found to have both synergistic and antagonistic effects on cellular functions resulting in alterations in function and metabolism. The effect of the isomers has been notably different between strains of animals and species, where CLA is primarily associated with advantages to health showing reduced adiposity, improved metabolism of plasma lipoprotein [54], insulin sensitivity [55] and decreased atherosclerosis [56]. Unfortunately, not all of these health benefits in animal models have translated well into clinical studies investigating the effects of CLA on human health. However, CLA blends enriched in c9,t11 and t10,c12 isomers have been identified as safe and effective in humans [52].

CLA dietary supplementation, using a 50 : 50 blend of c9, t11 : t10 , c12-CLA, has shown reductions in fat mass in both overweight and obese adults and children [57, 58], increased HDL cholesterol and decreased the ratio of LDL : HDL cholesterol in type 2 diabetes [59], diminished incidence of atherosclerosis in sedentary young adults [60]. Also c9,t11-CLA supplementation lowered the risk of CVD in men [61] and it was found that women who consumed four or more servings of high fat dairy foods rich in CLA reduced their risk of developing distal colon cancer by 34%, when compared with women who consumed less than one serving per day [49]. There was also a 35% reduction in the risk of colorectal cancer (CRC) in women who consumed at least three servings of cheese day^{-1} [49]. Noone *et al*. investigated the effects of CLA on cardiovascular disease risk factors in 51 healthy human subjects. In this 8 week, randomized, double-blind placebo study, c9,t11-CLA and t10,c12-CLA isomers were investigated using linoleic acid as the control. The group receiving the 50 : 50 CLA blend showed significantly decreased fasting plasma triacylglycerol (TAG) concentrations. Elevated plasma TAG concentrations are a risk factor of ischaemic heart disease [62]. Very low density lipoprotein (VLDL) cholesterol concentrations were significantly reduced in the group receiving the 80 : 20 CLA blend [63]. The effects of CLA supplementation on the immune system were investigated in 28 young healthy volunteers. In this 12 week study, volunteers received a dietary supplement of 3 g day^{-1} of c9, t11 : t10,c11-CLA blend (50 : 50). In CLA supplemented volunteers there was a significant increase in the anti-inflammatory cytokine IL-10, a decrease in pro-inflammatory cytokines TNF-α and IL-1β and a decrease in delayed-type hypersensitivity (DTH) response [64]. Interestingly in a separate study in humans with birch pollen allergy, c9, t11-CLA supplementation (2 g day^{-1}, 12 weeks) significantly reduced granulocyte M-CSF (GM-CSF), a known driver of the pro-inflammatory MΦ1 phenotype [65]. However, other studies have shown there to be no change in body composition [66] or immune function [67] following CLA supplementation. There is a need for further rigorous clinical investigation into the benefits of CLA supplementation and for characterization of the optimum blend of CLA to use in humans.

Effects of CLA on inflammation

In vivo studies have demonstrated that CLA has effects on diseases with an inflammatory component. In murine studies administration of t10,c12-CLA resulted in body composition changes most notably a reduction in body fat [68]. CLA treatments in AKR/J mice also reduced body fat via a reduction in energy intake and a reduction in metabolic rate [69]. Further studies in AKR/J mice also demonstrated a rapid decrease in fat accumulation on mice fed relatively low doses of CLA [70]. In Zucker diabetic fatty (ZDF) rats, c9,t11-CLA and c10, t12-CLA isomers in a 50 : 50 blend given as a dietary supplement improved glucose tolerance and decreased adiposity [71]. This same 50 : 50 CLA blend was used as a dietary supplement in a pig model of dextrin sodium sulfate (DSS)-induced colitis. Dietary CLA supplementation induced the up-regulation of peroxisome proliferator activated-receptor (PPAR)-γ expression and was found to delay the development of DSS-induced colitis [72]. Further studies indicate that PPAR-γ activation causes the down regulation of effector CD4$^+$ T cell function which is the primary mediator of colitis [73]. In addition to the PPAR-γ-dependent effects of CLA, studies in mouse models of DSS-induced colitis and CD4-induced colitis have shown that CLA blend (50 : 50 blend of c9,t11-CLA and t10,c12-CLA isomers) repressed TNF-α and nuclear factor-kappa B (NF-κB) activation and induced the expression of PPAR-γ coactivator 1-alpha (PGC-1α) and TGF-β1 [74]. Mice with inflammation induced CRC were fed a 50 : 50 blend of the c9,t11-CLA and t10,c12-CLA isomers. Through activation of PPAR-γ the development of the CRC was ameliorated thus demonstrating the chemoprotective properties of CLA on gut malignancies. Tumor development and colonic TNF-α were also suppressed in mice on the CLA diet [75]. Together this suggests that CLA isomers have positive physiological effects on diseases which have an inflammatory component.

Effect of CLA on atherosclerosis

The two most abundant CLA isomers have been shown to have anti-atherogenic effects in an experimental model of atherosclerosis when administered in an 80 : 20 blend [76]. CLA has been shown to induce the regression of atherosclerosis in mice, rabbits and hamsters. However, Arbonés-Mainar *et al*. demonstrated that different CLA isomers have different atherogenic effects. ApoE$^{-/-}$ mice fed on a Western diet (0.15% cholesterol) were supplemented with either c9, t11-CLA, t10,c12-CLA or linoleic acid (control diet) for 12 weeks. Development of atherosclerotic lesions was impaired in mice fed with c9,t11-CLA. In contrast, pro-

atherogenic effects were observed in the mice receiving t10, c12-CLA. Some of these effects included hyperlipidaemia, elevated macrophage content and activation and high plaque vulnerability in comparison with controls [77]. In the Belton group, we have previously demonstrated that the c9,t11 : t10, c12-CLA (80 : 20) blend not only inhibited the progression of atherosclerosis but induced regression of the lesions in a mouse model of atherosclerosis. Atherosclerosis was induced in apoE$^{-/-}$ mice through administration of a 1% cholesterol diet for 8 weeks. Mice were continued on the diet supplemented with 1% saturated fat (control) or 1% CLA blend (c9,t11 : t10,c12-CLA 80 : 20) for a further 8 weeks. Mice fed the supplemented CLA diet displayed almost complete regression of aortic atherosclerotic lesions. In comparison with controls, CLA-fed mice also had decreased aortic macrophage accumulation and decreased expression of CD36 [76]. Increased expression of PPAR-α and PPAR-γ within the aortas and the negative regulation of pro-inflammatory gene expression were also detected suggesting that CLA exerts its pro-resolving effects at least in part via activation of PPARs [76]. In a study by Lee *et al*. rabbits were put on a diet rich in fat (14% fat and 0.1% cholesterol) for 22 weeks in the presence or absence of 0.5 g of CLA day^{-1}. In the CLA fed group LDL concentrations were markedly lower and there was less evidence of atherosclerosis [54]. In a separate study it was shown that CLA-fed hamsters had reduced concentrations of plasma total cholesterol, LDL and triglycerides in comparison with the control group and also had less early atherosclerotic lesion development [56]. Together the data from *in vivo* studies suggest that CLA mediates its effects in atherosclerosis via inhibition of the inflammatory response and by modulation of circulating cholesterol.

CLA modulates monocyte/macrophage function

As described above monocytes and macrophages play a pivotal role in atherosclerotic lesion initiation and development. Our work has shown that in the apoE$^{-/-}$ model of atherosclerosis, CLA promotes a pro-resolving microenvironment [76], and our work and that of others have identified that the monocyte/macrophage is the cellular target through which CLA mediates regression of atherosclerosis *in vivo* [76].

Dysfunctional endothelial cells and subsequent monocyte recruitment is a hallmark in the pathogenesis of atherosclerosis [78]. VLA-4 and Mac-1 are key adhesion ligands on monocytes that bind VCAM-1 and ICAM-1, respectively, on endothelial cells, allowing for monocyte adhesion to the vessel wall [23, 24]. It has been shown that c9,t11-CLA and t10, c12-CLA isomers limited VLA-4 and Mac-1 expression on monocytes [79]. VCAM-1 and ICAM-1 expression on endothelial cells was only reduced after treatment with the c9, t11-CLA isomer [79]. This suggests CLA can modulate the adherence of monocytes to endothelial cells, unfolding a potential protective mechanism in early atherosclerosis.

Accumulation and migration of monocytes and macrophages arises due to elevated levels of MCP-1, and this has been reported in atherosclerotic plaques [80]. It has been shown that mice deficient in MCP-1 have significantly reduced atherosclerosis [81], highlighting the importance of monocyte migration as a critical step in the development of the disease.

CLA has been shown to inhibit monocyte migration to MCP-1 *in vitro* via a PPAR-γ dependent mechanism [82]. However, CCR2, the receptor for MCP-1, was not affected by CLA [82]. Subsequent studies showed that CLA inhibits the production of MCP-1, explaining the reduced migratory phenotypes of CLA-treated macrophages [82]. This implies CLA is a potent inhibitor of monocyte migration *in vitro* and may have promise in combating migratory pathogenic monocytes in atherosclerosis.

Macrophages of both a 'classical' MΦ1 and 'alternative' MΦ2 phenotype have been found in human atherosclerotic lesions [83]. It has been shown that the MΦ1 macrophage content of atherosclerotic plaques is associated with the clinical incidence of ischaemic stroke and increased inflammation or fibrinolysis [84]. In the context of atherosclerosis, it has been shown that there is an MΦ2 to MΦ1 switch during plaque progression. This is likely due to a conversion of cells already present in the lesion, suggesting that interventional tools, able to revert the macrophage infiltrate towards the MΦ2 phenotype, may exert an atheroprotective action. We have shown that CLA primes monocytes towards a pro-resolving MΦ2 macrophage [85]. The 80 : 20 blend of c9, t11 : t10,c12-CLA impacted on macrophage polarization by reducing expression of the MΦ1 macrophage marker CD68 [85] and increasing expression of CD163 and mannose receptor, receptors associated with the MΦ2 anti-inflammatory phenotype, in human peripheral blood mononuclear cell (PBMC)-derived macrophages [85]. Interestingly, this effect was mediated in part via a PPAR-γ dependent mechanism. These data supports the findings of a previous study carried out by Bouhlel *et al*. where PPAR-γ activation was associated with MΦ2 differentiation and increased the expression of the mannose receptor MΦ2 marker in PBMCs [86]. In another study, IL-1RA was upregulated in CLA-treated RAW 264.7 macrophages and this correlated with decreased secretion of IL-1α, IL-1β and IL-6 pro-inflammatory MΦ1 cytokines [87]. This effect in RAW 264.7 macrophages was only observed using the c9,t11-CLA isomer [87]. We have confirmed these findings *in vivo* where we showed that CLA supplementation in apoE$^{-/-}$ mice induced the anti-inflammatory MΦ2 phenotype via increasing IL-10 production in atherosclerosis regression (88). Together this suggests that CLA primes the monocyte/macrophage towards a pro-resolving MΦ2 phenotype to exert atheroprotective effects. However, comprehensive characterization of CLA treated monocytes and macrophages warrants further investigation, specifically in the context of macrophage plasticity.

CLA limits foam cell formation

Uptake of oxLDL by macrophages, via scavenger receptors CD36 and SR-A1, is important for foam cell formation and subsequent fatty streak development [89]. CLA promotes atheroprotection, by inhibiting foam cell formation and regulating expression of RCT genes, thus enhancing the removal of cholesterol from the circulation [90, 91]. Documented

targets of CLA include the nuclear receptor liver X receptor alpha (LXRα) and cholesterol efflux genes such as ABCA1 and ABCG1 [91, 92]. It has been shown that c9,t11-CLA acts as a direct agonist for LXRα [92]. We have also verified this, showing that CLA inhibits foam cell formation *in vitro* via a PPAR-γ- and LXRα-dependent mechanism [85].

CLA reduces inflammatory mediators

Fatty acids have been found to decrease the inflammatory output of the macrophage, which shows the potential therapeutic benefit of CLA in targeting the inflammatory component of atherosclerosis [56, 93, 94]. In RAW 264.7 macrophages and PBMCs stimulated with LPS, a potent activator of the inflammatory pathway, NF-κB, as well as prostaglandin E_2 (PGE_2) and cyclo-oxygenase-2 (COX-2) were attenuated upon treatment with both c9:t11-CLA and t10, c12-CLA [95, 96]. The result indicates that CLA has the capacity to act as an anti-inflammatory modulator of monocytes and macrophages. Similarly Yu *et al*. used a 50 : 50 blend of the c9,t11-CLA and t10,c12-CLA isomers and found a reduction in inducible nitric oxide synthase (iNOS) and PGE_2 in RAW 264.7 macrophages [97]. This study also highlighted the importance of CLA as an anti-inflammatory lipid as it revealed a reduction in the expression of the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 [97]. These mediators are hallmarks of the pro-inflammatory macrophage phenotype (MΦ1), characteristic of atherosclerosis progression.

IL-10, a potent anti-inflammatory cytokine, has been identified in atherosclerotic plaques and is predominantly secreted from macrophages [98]. Decreased levels of iNOS, an MΦ1 macrophage marker, were associated with high levels of IL-10 expression and, inversely, plaques with elevated iNOS correlated with minimal IL-10 expression and more frequent cell death [98], indicating a strong association of cell death with IL-10 expression. Studies investigating the effects of CLA on IL-10 to date have been sparse. However we have demonstrated *in vivo* that components of the IL-10 signalling pathway were modified with CLA treatment [88]. We have reported increased IL-10 receptor expression, increased phosphorylation of signal transducer and activator of transcription 3 (STAT3) in the aorta and an increase in serum IL-10 concentrations in CLA-induced regression (88). Furthermore, CLA-induced IL-10 production diminished TNF-α concentrations thus contributing to regression of pre-established atherosclerosis [88]. Thus CLA impacts on macrophage phenotype and inflammatory mediators enabling regression of established plaques in atherosclerosis models.

CLA as an agonist for PGC-1α

CLA, as mentioned, inhibits monocyte/macrophage adhesion, migration, differentiation of classical macrophages, foam cell formation and generation of inflammatory mediators via a PPAR-γ dependent and independent mechanism. To elucidate if there was a common mechanism regulating the aforementioned atheroprotective effects of CLA we performed transcriptomic analysis of the aorta from CLA fed $apoE^{-/-}$ mice. This study identified several 'hub genes' regulated by CLA, most notably PGC-1α [90]. PGC-1α is a transcriptional co-activator of several nuclear receptors and has recently been established as a mediator in atheroprotection *in vivo* [90]. In $apoE^{-/-}$ PGC-1α gene expression was localized to the macrophage cells of the aorta. CLA supplementation increased PGC-1α gene expression and, interestingly, expression was inversely correlated with lesion burden [90]. Furthermore, in RAW 264.7 macrophages treated with CLA and oxLDL there was increased PGC-1α expression and increased expression of PGC-1α target genes, namely uncoupling protein 1 (UCP-1) and cytochrome P450, family 7, subfamily B1 (CYP7B1) [90]. PGC-1α expression was associated with decreased oxLDL uptake and decreased foam cell formation [90]. The importance of macrophage PGC-1α in atherosclerosis was confirmed when macrophage specific deletion of PGC-1α accelerated atherosclerosis in the LDL $receptor^{-/-}$ ($LDLR^{-/-}$) mouse [90]. Finally, we localized PGC-1α to macrophages and foam cells of human atherosclerotic plaques and showed that its expression was inversely associated with disease progression. This suggests that the molecular mechanism through which CLA mediates the resolution of atherosclerosis is via regulation of PGC-1α.

Conclusion

Macrophage accumulation and foam cell formation after lipid acquisition is a classic hallmark of atherogenesis. While there is much information on the cellular and molecular mediators in the pathogenesis of atherosclerosis there is limited information on the pathways involved in disease regression. The implications of this are important since most patients present with pre-established lesions and the therapeutic goal would be to reverse the lesion. Our work and that of others has shown that a specific blend of CLA isomers, not only inhibits progression, but induce regression of pre-established atherosclerosis in $apoE^{-/-}$ mice. Furthermore, it is now established that the monocyte/macrophage is the cellular target through which CLA mediates its effect. It has been shown both *in vitro* and *in vivo* that CLA inhibits monocyte adhesion, monocyte migration and inhibits uptake of LDL cholesterol by macrophages [90–92]. The atheroprotective effects of CLA have facilitated identification of a number of potential mechanisms underlying regression and the data suggest that CLA is a pro-resolving lipid mediator which alters the lesion microenvironment. The effects of CLA isomers on the development, progression and resolution of atherosclerosis continue to be thoroughly investigated to yield further information as to how CLA modulates and induces regression of atherosclerosis.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organizations for the submitted work, no financial relationships with any organizations that might have an

interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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