Postprandial lipoproteins and the molecular regulation of vascular homeostasis

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Abstract

Blood levels of triglyceride-rich lipoproteins (TRL) increase postprandially, and a delay in their clearance results in postprandial hyperlipidaemia, an important risk factor in atherosclerosis development. Atherosclerosis is a multifactorial inflammatory disease, and its initiation involves endothelial dysfunction, invasion of the artery wall by leucocytes and subsequent formation of foam cells. TRL are implicated in several of these inflammatory processes, including the formation of damaging free radicals, leucocyte activation, endothelial dysfunction and foam cell formation. Recent studies have provided insights into the mechanisms of uptake and the signal transduction pathways mediating the interactions of TRL with leucocytes and vascular cells, and how they are modified by dietary lipids. Multiple receptor and non-receptor mediated pathways function in macrophage uptake of TRL. TRL also induce expression of adhesion molecules, cyclooxygenase-2 and heme-oxygenase-1 in endothelial cells, and activate intracellular signaling pathways involving mitogen-activated protein kinases, NF-κB and Nrf2. Many of these effects are strongly influenced by dietary components carried in TRL. There is extensive evidence indicating that raised postprandial TRL levels are a risk factor for atherosclerosis, but the molecular mechanisms involved are only now becoming appreciated. Here, we review current understanding of the mechanisms by which TRL influence vascular cell function.

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1. Introduction

Atherosclerotic cardiovascular disease is a primary cause of death worldwide [1] and the single most common cause of death in developed countries [2,3]. The development of atherosclerotic lesions begins with dysfunction of the vascular endothelium, followed by activation and recruitment of monocytes to the artery wall where they differentiate into macrophages and take up cholesterol and other lipids from the plasma lipoproteins to form foam cells. The accumulation of these lipid-engorged cells, together with the proliferation of vascular smooth muscle cells (VSMC), causes fatty streaks, the first visible arterial lesions, to appear [4,5]. It has been known for many years that low density lipoprotein (LDL), particularly when oxidatively modified, plays a major role in atherosclerosis initiation and development [6], and the molecular mechanisms involved in its effects have been studied extensively [7–9]. In recent years, however, it has become clear that postprandial lipemia, which is caused by the raised levels of triglyceride-rich lipoproteins (TRL) present in the blood after a meal containing fat, is also a risk factor for the disease [10–12]. Postprandial lipemia is induced by fat meals containing >30 g fat [13,14], and, given that on average in Western countries 3–4 meals containing of 20–40 g fat are consumed each day, it has been estimated that raised levels of TRL may persist for 18 h per day in these populations [15].

After absorption in the intestine, dietary lipids are packaged into large TRL called chylomicrons (CM), and secreted into lymph and ultimately into the blood via the thoracic duct. Lipolysis by lipoprotein lipase (LPL) in extrahepatic capillary beds then converts the CM to smaller, but still triglyceride-rich chylomicron remnants (CMR) which are cleared from the circulation by the liver [16,17]. The fatty acids derived from the TG delivered in CMR may either be used by the liver or re-synthesized into new TG and returned to the blood (together with cholesterol and phospholipid) in very low density lipoprotein (VLDL) [18]. Thus, blood levels of CM, CMR and VLDL all contribute to postprandial lipemia [19] and these lipoproteins are collectively known as TRL. In humans, TRL derived from the intestine (CM and CMR) contain apolipoprotein (apo) B48, while those derived from the liver (VLDL) contain apoB100 [16,19].

It has become clear over the past two decades that atherosclerosis is a chronic inflammatory disease. Inflammatory processes have been shown to be important in both the initiation and progression of lesion development [20,21]. Evidence indicates that postprandial lipemia is pro-inflammatory, with each meal causing a transient change, known as postprandial metabolic inflammation [12,22], and TRL are thought to play a major role in a number of the inflammatory processes involved, including the excessive formation of damaging free radicals, leukocyte activation, endothelial dysfunction and foam cell formation [12,23,24].

Since experimental, epidemiological and clinical evidence for considering non fasting TRL as a risk factor for atherosclerosis is now very strong [10–12,14,22–27], a better understanding of the molecular events by which they influence lesion initiation and development is of great importance. In this article, we review recent studies which have begun to delineate the receptors and signal transduction pathways mediating the potentially atherogenic interactions of TRL with circulating leukocytes and cells of the vasculature, and how they are influenced by dietary components carried in the particles, including the type of fat (saturated, unsaturated or oxidized) and other micronutrients.

2. Postprandial lipoproteins and atherosclerosis

Zilversmit first suggested that postprandial lipemia may play a role in atherogenesis about thirty years ago [28]. Although this idea was slow to gain acceptance, in recent years a great deal of epidemiological, clinical and experimental evidence has accumulated to support the proposal, and there is now a consensus that non-fasting TRL levels are a clinically significant risk factor for atherosclerosis and the progression of cardiovascular disease [12,29–33]. Atherosclerosis progression has been linked with delayed clearance of TRL [12,34–36] and postprandial hyperlipidemia is implicated in the increased risk of premature atherosclerosis in patients with common metabolic conditions such as obesity, diabetes and the metabolic syndrome [26,37–39]. Furthermore, in two Japanese studies involving sudden death cases, the majority of cardiac deaths were found to be associated with postprandial hyperlipidemia and plasma remnant lipoprotein levels rather than with LDL cholesterol, leading the authors to propose that plasma remnant lipoprotein concentration is a major pathological factor in cardiovascular events [40,41]. Supporting evidence is also provided by three recent large scale prospective studies, which reported an association between non-fasting plasma TG levels and the incidence of cardiovascular events which was independent of other risk factors [10,42,43]. Postprandial hyperlipidemia or increased plasma TRL levels have also been shown to be related to increased thickness of the carotid artery intima in humans [44,45] and in experiments with a rabbit model of postprandial hypertriglyceridemia [46].

The role of TRL as a risk factor for atherosclerosis is also supported by the concept of ‘residual risk’ [39]. Statin therapy aimed at lowering LDL cholesterol reduces cardiovascular events by about one third, leaving a residual two thirds which do not appear to be related to LDL, and the ‘residual risk’ is greater for subjects undergoing treatment for diabetes or the metabolic syndrome than in healthy individuals [47,48]. Thus, it has been proposed that the ‘residual risk’ of atherosclerosis is dependent on plasma remnant lipoprotein concentrations in addition to LDL cholesterol [39].
TRL may promote atherosclerosis indirectly by predisposing to a more atherogenic lipoprotein profile when their levels are raised by overproduction or delayed clearance [14,29,31,49]. Postprandial hyperlipidemia has been linked to a rise in the proportion of small, dense LDL [50–53], which enter the vessel wall more easily than larger LDLs, are more prone to oxidation, bind more strongly to the arterial wall and are cleared at a slower rate, and are thus more atherogenic [50,54]. Delayed TRL clearance also provides increased opportunity for the transfer of lipids from high density lipoprotein (HDL) leading to a lowering of HDL cholesterol [31]. Raised plasma TG and small dense LDL and lowered HDL is known to be an atherogenic lipoprotein profile and is termed the lipoprotein lipid triad [55].

As well as the indirect effects of TRL on atherosclerosis, current evidence suggests that they directly promote initiation and progression of lesion development by influencing events in the blood and vessel wall [12,14,17,37,49]. It has been demonstrated that remnant-like lipoproteins (RLP) are present in human atherosclerotic plaque [56–58], that CMR enter and are retained in the artery wall [59–61], and that CMR and TRL cause endothelial dysfunction [27,37,62–64], macrophage foam cell formation [37,65] and VSMC proliferation [37]. In addition, TRL have been shown to activate leukocytes, [29,66], and to cause pro-thrombotic effects, including a rise in platelet aggregation, coagulant factor VII [68] and plasminogen activator inhibitor type 1 (PAI-1) [31,67–70]. Clearly, increased accumulation of TRL in the blood, either because of overproduction or delayed clearance, is likely to enhance their direct atherogenic effects.

Prior to their clearance by the liver, TRL are metabolized by LPL while bound to endothelial cell surfaces via hepatic sulfate proteoglycans (HSPG) in extracellular tissues. The recent discovery of two new proteins, lipase maturation factor 1 and glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (GPIHBP1), has led to significant progress in understanding the molecular mechanisms regulating these processes (recently reviewed in detail elsewhere, [71–75]), which must function efficiently to avoid the development of postprandial hyperlipidemia.

Although it has been clear for many years that oxidation of LDL greatly enhances its atherogeneity [6], much less attention has been paid to the effects of oxidation on the increased risk of lesion development associated with TRL. However, lipids carried in TRL may become oxidized in the artery wall by the cell-associated lipoprotein and myeloperoxidase enzymes which are responsible for LDL oxidation, and oxidized lipids which are present in food after fat is cooked at high temperatures are known to be trans-located to LDL, resulting in the formation of oxidized lipids which are metabolized by the liver, in CM. These lipids are then transferred to CMR, which contain VSMCs. Thus, LDL oxidation may cause TRL to be trapped in the artery wall, leading to the development of atherosclerosis.

3. Postprandial lipoproteins and molecular regulation of vascular smooth muscle cell function

Switching of VSMCs from a contractile and quiescent to an inflammatory and migratory phenotype plays a key role in the pathological vascular remodeling associated with atherosclerosis and is characterized by trans-differentiation culminating in markedly heightened proliferative, migratory and secretory functions which accelerate inflammation. There is limited evidence from in vitro studies using VSMCs that exposure to endogenous oxidized lipoproteins (principally oxLDL or oxphospholipid (oxPL) can facilitate the onset of these phenotypic changes as well as promote osteogenic differentiation which contributes to VSMC calcification in plaques (see [37,81]). With respect to postprandial TRL, a series of studies has made use of CMR isolated from the plasma of hepatectomized rats and examined the actions of these ApoE-containing particles on cultured rat aortic ECs [82,83]. Incubation with CMR, but not with CM or oxLDL, increased monocyte chemoattractant protein (MCP)-1 mRNA expression (but not interleukin (IL)-1β or tumour necrosis factor (TNF)-α) in rat VSMC [82,83] and experiments with pharmacological inhibitors of p38 mitogen-activated protein kinase (p38MAPK) and mitogen activated protein kinase kinase (MEK) suggested that this occurs in a p38MAPK-dependent manner but does not require activation of the MEK- extracellular-signal-regulated kinase (ERK) pathway [82]. Conversely, ERK1/2 activation triggered by rat CM seems to regulate expression of the transcription factor early growth response protein (Egr)-1 [83], which is strongly implicated in promoting inflammatory activation of both VSMCs and ECs [84], whereas this response occurs independently of p38MAPK [83]. RLP from patients with hypertriglyceridemia have been shown to promote rat aortic smooth muscle cell proliferation through PKC-mediated transactivation of the epidermal growth factor (EGF) receptor [85], suggesting that, in common with EC exposure to oxPL (see above) this mode of signaling interaction may also be important for determining VSMC responses to TRL. It should be noted that while these studies provide some evidence for proatherogenic modulation of VSMCs by CMR, they are confined to rodent ECs so the importance of the documented changes in VSMC signaling and function for human vascular smooth muscle awaits clarification.

There is nothing known about how the FA composition of TRL/CMR influences VSMCs, but it is of note that the n-3 PUFA, docosahexaenoic acid (DHA) has been shown to limit IL-1β-stimulated VSMC migration and proliferation, and reduces both matrix-metalloproteinase (MMP) activity and plasminogen activator inhibitor type 1 (PAI-1) secretion [86]. These effects seem to be dependent upon altered expression of Notch signaling components, a pathway which is now strongly implicated in VSMC phenotypic modulation. Precisely how TRL of different FA composition influence VSMC function is unexplored and now warrants investigation.

4. Postprandial lipoproteins and molecular regulation of endothelial cell function

As indicated above, TRL present in the circulation during the postprandial phase comprise VLDL, CM and their remnants and are complex particles composed of TAG, phospholipids, cholesterol, cholesterol esters and protein (see [37]). During the postprandial period, CM are produced from exogenous lipids and since newly synthesized TG are incorporated preferentially into these particles their fatty acid composition directly reflects that of the diet. Lipid soluble micronutrients are also incorporated into CM during their formation in the gastro-intestinal tract. Thus, it should be borne
in mind that their effects on the vascular wall in vivo will be dictated by their overall composition and potentially reflect the combined actions of a number of components.

4.1. Endothelial dysfunction

The endothelium is a cell monolayer lining the luminal surface of all blood vessels and regulates the movement of cells, proteins and other components between the circulation and the extravascular compartment. Endothelial cells (EC) respond to diverse paracrine, autocrine and endocrine signals with changes in cell surface protein expression and mediator secretion which allows the endothelium to regulate a range of pathophysiological processes including inflammation, thrombosis, vasomotor tone, angiogenesis and cancer cell metastasis (see [25–27]). In health, the complex molecular mechanisms controlling EC responses to extracellular stimuli render the endothelium anti-inflammatory and anti-thrombotic, thus ensuring vascular homeostasis. In contrast, disruption of these mechanisms by numerous factors (e.g. increased circulating inflammatory mediators) leads to a persistent state of endothelial activation and inflammation which is characterized by overproduction of reactive oxygen species (ROS), impaired generation of vasodilatory mediators (e.g. nitric oxide) and increased expression of adhesion molecules. Collectively, this state of endothelial dysfunction results in altered communication between endothelial cells, VSMCs and circulating cell components and facilitates monocyte adhesion/migration as well as phenotypic alteration of VSMCs. These inflammatory changes are associated with impaired endothelium-dependent vasodilatation and precede the onset of pro-atherogenic alterations in the vascular wall, thus placing 'inappropriate' endothelial cell activation at the center of the vascular events initiating the development of atherosclerotic disease.

The literature describing the endothelial effects of endogenous lipoproteins, primarily modified and native forms of LDL and HDL, is extensive and has been comprehensively reviewed elsewhere [4–6,20]. In marked contrast, there is currently little understanding of how exogenous lipoproteins that carry fat from the diet influence endothelial function in health and disease. Altered responsiveness of ECs to the actions of these particles is likely to be critical in determining the extent of endothelial dysfunction and thus in influencing leukocyte–endothelial interactions and the pro-atherogenic consequences of these inflammatory changes.

4.2. The endothelial glycocalyx: potential influence on TRL action

Retention of lipoproteins within the subendothelium as a consequence of endothelial dysfunction is considered to be an important early event in the initiation of atherosclerosis (reviewed in [87]). In vivo, the luminal surface of the vascular endothelium is decorated by a specialized extracellular matrix (the glycocalyx) composed of glycoproteins, glycosaminoglycans and adsorbed plasma proteins. The normal unperturbed luminal glycocalyx provides a barrier to transendothelial or transvascular leakage of lipoproteins and limits adhesion of circulating platelets and leukocytes to the endothelium (see [88]). Thus, its presence or absence is likely to impact significantly on the ability of circulating lipoproteins to interact with the vascular wall. In keeping with this, reduced thickness and/or disorganization of the arterial glycocalyx (e.g. in atheroprone regions of the arterial circulation) is linked with increased susceptibility to ‘leakage’ of LDL across the endothelium and subsequent accumulation of these lipoproteins within the arterial intima [89]. Endothelial dysfunction and disorders facilitated by this condition (e.g. atherosclerosis) are characterized by increased production of inflammatory cytokines (e.g. TNF-α) which have been reported to impair the overall barrier properties of the glycocalyx, potentially through reactive oxygen species (ROS)-mediated degradation of its components (see [88]); thus, inflammation and the associated reduction in glycocalyx dimensions, together with changes in permeability of the endothelial cell monolayer itself, could help to explain the increased intimal deposition accumulation and arterial retention of apoE- and apoB48-containing lipoproteins in human vessels and vessels from experimental animals with hypercholesterolaemia [60,90] (see [91] for review).

Despite the fact that the microcirculation becomes dysfunctional in cardiovascular disorders (see [92,93]) there is relatively little known about how the capillary luminal glycocalyx influences lipoprotein interactions. In this respect, recent studies in mouse cremaster muscle have shown that high fat feeding-induced hyperlipidaemia is associated with reduced capillary glycocalyx dimensions together with subendothelial accumulation of lipoproteins with a size suggestive of chylomicrons [94]. Additional experiments with labeled chylomicrons showed that partial destruction of the glycocalyx by infusion of heparinase promoted subendothelial deposition of the labeled particles [94], indicating that the microvasculature is also susceptible to hyperlipidaemia-induced changes in glycocalyx dimensions and that this is accompanied
by transcellular and/or paracellular movement of chylomicrons (and presumably CMR) across the endothelium. The observation that TRL accumulate within the subendothelium under these pro-inflammatory conditions offers ample opportunity for these particles to interact directly with the luminal and abluminal EC membranes, as well as with junctional complexes, and thus to influence EC function. It is also possible, however, that in vivo CM and CMR may be retained within the luminal glycoalyx, since a proteoglycan and glycosaminoglycan binding site has been identified in the amino terminal region of apoB48 [95,96]; this could enhance activation of arterial or capillary endothelium by CMR by facilitating prolonged contact with the EC surface and thus increasing the probability of interaction through receptor binding or other mechanisms. Whether and by what mechanisms microvascular EC function is affected by CM or their remnants is not known, but given the documented actions of these lipoproteins on large vessel ECs (see below) it is conceivable that these particles are also capable of influencing inflammatory and anti-inflammatory processes in microvascular ECs; this is an unexplored research area which may shed light on the specific actions of these particles within the microcirculation. It should be noted, however, that the large number of in vitro studies investigating lipoprotein effects on ECs most probably provide information that reflect their actions in conditions of reduced glycoalyx dimensions since current evidence suggests that cultured ECs do not elaborate a glycoalyx that resembles that present in vessels in vivo (see [88]).

Non-enzymatic peroxidation of PUFA esterified in PL generates PL containing a mixture of oxidized fatty acid residues (oxPL). These oxPL are active components of oxLDL and are also present in the membranes of cells subjected to oxidative stress and undergoing apoptosis [97]. Since the processes resulting in lipoprotein oxidation in vivo are comparable for all lipoprotein classes (see [98]) it is highly likely that CMR will also contain oxPL species [99], although the levels of these would be predicted to be lower in CMR, reflecting their lower PL content. Reports detailing the effects of oxidized versus unmodified CMR on ECs are currently lacking, but our recent data indicate that this modification enhances the ability of CMR to activate a number of intracellular signaling pathways (ERK1/2, Akt and p38MAPK), and to modify expression of pro-inflammatory and anti-oxidant genes in human ECs (100, Dalla-Riva et al., unpublished). Thus, oxidative modification of PL carried in exogenous lipoproteins may have important functional consequences for the vascular wall during the postprandial phase, a hypothesis which remains to be tested directly.

A wealth of studies have examined the actions of exogenously added oxPL on endothelial cells in vitro with oxidized 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphocholine (oxPAPC) the most commonly used representative species (see [98]). There is now a large body of evidence demonstrating that these modified lipids exert both detrimental (pro-inflammatory) and protective (anti-inflammatory/anti-oxidant) effects on ECs mediated through receptor-dependent and receptor-independent mechanisms. Interestingly, recent studies suggest that the concentration of oxPL is a critical determinant of their cellular actions with low levels inhibiting LPS-mediated E-selectin expression in human umbilical vein endothelial cells (HUVEC) and ten-fold higher concentrations promoting release of the pro-inflammatory cytokine IL-8 [101]. The authors hypothesize that low circulating levels of oxPL limit Toll-like receptor-4 (TLR-4)-dependent inflammatory responses in ECs and other cells, whereas the pro-inflammatory effects may be more relevant in situations characterized by high local concentrations of these mediators (e.g. within atherosclerotic plaques) [101]. These data are intriguing since they strongly suggest that oxPL have dual actions, promoting both pro- and anti-inflammatory effects on ECs, and indicate that the functional outcomes of exposure of ECs to these lipids is highly context-dependent. Similarly, CMR-like particles containing n-6 PUFA induce robust expression of cyclooxygenase-2 (COX-2) and downstream prostanoid production in ECs, activate ERK1/2 and p38MAPK and also suppress basal and agonist-stimulated cGMP formation, reflective of reduced nitric oxide (NO) production [100]. While these findings indicate that CMR are capable of upregulating inflammatory signaling and gene expression in ECs, these particles concomitantly induce expression of the anti-oxidant and anti-inflammatory cytoprotective enzyme heme oxygenase-1 (HO-1), suggesting dual functions for remnant lipoproteins in regulating endothelial homeostasis ([102], Dalla-Riva et al., unpublished). In this respect it is noteworthy that oxPAPC also increases HO-1 expression in aortic endothelium [103].

One factor that could explain the plethora of cellular actions attributed to oxPL is the range of receptor types with which they are reported to interact. For example, direct and indirect associations of these oxPL (or lipoproteins containing them) with growth factor receptors (e.g. VEGF receptor-2) on ECs has been documented, providing a mechanism through which lipoproteins could influence signaling important for cell growth and tissue repair. Since oxPL are present in plaques, such actions may have significance in the context of atherosclerosis where neovascularisation is associated with advanced unstable lesions. In this respect, treatment with oxPAPC stimulates sprout formation by cultured endothelial cell spheroids and this process seems to depend upon the autocrine actions of pro-inflammatory mediators, including IL-8 and products of COX-2 activity [104]. As yet, it is unclear whether these growth-promoting effects of oxPL have relevance for physiological repair processes involving angiogenesis. The precise signaling mechanisms mediating these responses have also been not defined but the effects are comparable to those of classical pro-angiogenic cytokines (e.g. VEGF, leptin) that trigger activation of MAPKs (ERK1/2, p38MAPK), nuclear factor-kB (NF-kB) and Akt in human ECs [105,106] and use these pathways to regulate COX-2 induction, eicosanoid production and downstream angiogenic functions [106]. The expectation is that oxPL associated with CM or their remnants could influence the actions of these particles on ECs but, to date, there is no published evidence to support the specific involvement of CMR in positively regulating EC proliferation or angiogenesis.

Early in vitro studies suggested that prolonged (>24 h) exposure to CMR-like particles extracted from egg solution-fed rats actively promoted human EC apoptosis [107]. Other work has since shown that, in common with oxLDL, extended incubation with RLP (defined as VLDL remnants plus some CMR) derived from the plasma of hyperlipidaemic patients decreased EC viability and that this was associated with increased DNA fragmentation [108]. Reduced EC survival in the presence of RLP or oxidized RLP (oxRLP) was accompanied by increased NAD(P)H oxidase (Nox)-dependent superoxide production and markedly enhanced release of the pro-inflammatory cytokines TNF-α and IL-1β, both of which were inhibited by an antibody against lectin-like oxidized low density lipoprotein receptor (LOX-1); the authors concluded that RLP, oxRLP and oxLDL all interact with human ECs in a LOX-1-dependent manner [108], thus promoting pro-oxidant signaling and increasing inflammatory activation. While this report indicated the likely importance of Nox-derived ROS in mediating the detrimental actions of ‘pathological’ RLP fractions, the study was performed in an infrequently used transformed HUVEC cell line so the significance of these findings for adult ECs is unclear. In addition, these findings are distinct from those in human aortic endothelial cells (HAECs) reported more recently [24]. In the latter study, HAECs were repeatedly exposed to TRL isolated from human plasma following ingestion of a meal typical of a Western diet (30% fat with 20% saturated fat). Prolonged repetitive (1–3 days) or acute (4 h) exposure to unmodified TRL had no direct pro-inflammatory effect on HAECs as assessed by a failure to enhance EC vas-
cular cell adhesion molecule-1 (VCAM-1) expression or monocyte adhesion to ECs. ‘Priming’ HAECs with TRL, however, lowered the threshold concentration for TNF-stimulated VCAM-1 and E-selectin expression with a concomitant upregulation of monocyte recruitment [24]. While TRL treatment had no effect on the activation state of p38MAPK, ERK1/2, Akt or NF-κB in HAECs, the TRL-mediated sensitization to inflammatory stimuli appeared to be dependent upon p38MAPK activity, since priming strongly up-regulated sustained TNF-induced p38MAPK phosphorylation and the priming effect, most likely mediated through the NF-κB pathway, was abrogated by pharmacological blockade of p38MAPK [24]. This would result in a scenario where repeated exposure to circulating TRL resulting from high fat ingestion would permit more rapid and extensive TNF-α-mediated nuclear translocation and transcriptional activation of NF-κB. These findings have yet to be confirmed and the relevance of priming for the actions of other pro-inflammatory stimuli established. Interestingly, native VLDL (but not oxVLDL) was shown to be the TRL component responsible for the priming effect with no apparent contribution by CM [24]. The specific effects of CM on aortic ECs have not been established but it is possible that these particles influence aortic EC function through mechanisms distinct from those used by VLDL.

Overall, the data discussed above suggest that ‘normal’ postprandial TRL do not, on their own, promote inflammatory activation of the endothelium. In contrast, other studies have provided good evidence for direct activation of ECs by TRL from both normolipidaemic individuals as well as those with type IV hyperlipidaemia [64]. Both types of TRL transiently increased phosphorylation of p38MAPK and ERK1/2 and augmented downstream expression of a range of pro-inflammatory genes in HUVEC, including VCAM-1, E-selectin, MCP-1 and IL-6, through enhanced transcriptional activation of NF-κB and CAMP response element binding (CREB) [64]. It is notable, however, that the EC responses to TRL from the hyperlipidaemic patients were generally more robust than those promoted by ‘normal’ TRL [64]. Similarly, in a high fat feeding study, ECs exposed to human postprandial (4 h) TRL elicited greater changes in p38MAPK, transcription factor activation (CREB) and adhesion molecule expression (VCAM-1, intercellular adhesion molecule-1 (ICAM-1)) than TRL isolated immediately after fat ingestion [109]. The authors concluded that these differential effects may relate to the impaired postprandial flow-mediated dilatation evident in individuals with hyperlipidaemic disorders [109]. The ability of TRL to enhance EC adhesion molecule expression is in keeping with the positive association between postprandial hypertriglyceridaemia and the level of circulating adhesion molecules [110]. ApoCIII and lipoproteins containing ApoCIII (e.g. VLDL) have also been reported to increase VCAM-1 expression in ECs through mechanisms dependent upon PKCβ and NF-κB [111], leading to an increase in monocyte adhesion (see below). Other studies have shown that oxVLDL generally promotes a greater inflammatory activation of HUVEC as compared to unmodified VLDL [112]. Thus, VLDL preferentially activated ERK1/2 and had marginal effects on ROS production whereas oxVLDL triggered activation of p38MAPK accompanied by markedly enhanced generation of ROS and decreased cell viability. A gene array analysis also seemed to suggest surprisingly distinct changes in gene expression elicited by VLDL versus oxVLDL [112] but since this analysis was performed on a stable hybridoma cell line (EAHy926), and not on ECs, the overall relevance of these differing activation profiles remains unclear.

There are also reports that TRL alter endothelial production of mediators involved in coagulation and fibrinolysis, suggesting that the potentially detrimental effects of these particles on ECs are not restricted to changes in inflammatory gene expression. For example, exposure of human aortic ECs to ‘fasting’ RLP from patients with type III hyperlipoproteinemia was shown to increase expression and activity of PAI-1 [113], a serine protease inhibitor that limits fibrinolysis. Similarly, PAI-1 mRNA in HUVEC can be upregulated by TRL isolates derived from both normal and hyperlipidaemic human plasma [64]. It is possible that these changes are regulated, at least in part, by Nox4-derived ROS, since treatment of HUVEC with pharmacological Nox inhibitors or specific Nox4 silencing with siRNA abrogated p38MAPK phosphorylation, NF-κB activation, PAI-1 promoter activity and PAI-1 secretion [114]. In addition, similar changes in PAI-1 expression, mediated by p38MAPK, were evident in ECs exposed to oxidized HDL indicating that so-called ‘dysfunctional’ HDL may also exert pro-thrombotic actions on the endothelium [115]. To date, there is little understanding of whether or how the CMR component of TRL contributes to their apparent pro-thrombotic influence on endothelium. In this respect, CMR isolated from the plasma of functionally hepaticoportal rats supplied with exogenous CM have been reported to increase PAI-1 secretion and mRNA expression in HUVEC, possibly by stimulating interactions with a VLDL response element within the PAI-1 promoter [116]. These in vitro effects were abrogated by treatment with an angiotensin-converting enzyme inhibitor [116] indicating that the detrimental actions of CMR on ECs may be functionally antagonized by a commonly used vasculoprotective drug. Since these were rodent remnants the significance of these findings for understanding interactions of human CMR with the endothelium is currently unclear, but these data, together with those of Norata and co-workers [115], provide some evidence that TRL, and possibly the CMR component, may also influence the pro-thrombotic status of the endothelium and hence contribute to the initiation and/or progression of endothelial dysfunction.

Thus, it would appear that there are some differences between the effects of ‘physiological’ versus ‘pathological’ TRL on ECs, at least in vitro, with particles from hyperlipidaemic patients targeting pro-inflammatory and probably pro-thrombotic processes. The question of whether physiological TRL present postprandially after high fat ingestion have direct inflammatory effects on ECs [64], or not [24], is still unresolved and requires clarification. It is possible that the differential effects reported in these studies reflect differences in TRL isolation procedures, relative proportions of CM, VLDL and their remnants, lipoprotein concentrations used and EC types employed (adult versus fetal) [see [26]]. Nevertheless, these studies collectively highlight the potential importance of TRL in the postprandial phase as modifiers of endothelial (dis)function.

Pro-atherogenic inflammation seems to be a characteristic response of ECs in vitro to treatment with TRL and provides evidence to support the involvement of these lipoproteins in driving atherosclerotic changes in the vascular wall. Stress-induced premature senescence of ECs is also a factor driving endothelial dysfunction and can contribute to the impaired vascular repair capacity associated with chronic inflammation and aging by negatively influencing endothelial progenitor cell (EPC) function. In this respect it is worth noting that RLP isolated from the plasma of hypertriglyceridaemic subjects have been reported to accelerate EPC senescence [117,118]. EPCs isolated from peripheral blood showed increased nitrotyrosine staining following incubation with RLP, indicative of oxidative stress-associated production of peroxynitrite, and these effects were coupled to reduced proliferation and migration capacities and to reduced telomerase activity [117,118]. These findings strongly suggest that the effects of TRL on the vasculature are not restricted to direct actions on differentiated ECs and could well extend to progenitor cell populations important for tissue repair.

4.3. Receptor-dependent interactions of TRL with endothelium

As discussed below receptor-mediated interactions of TRLs, including CMRs, with macrophages are now reasonably well
characterized (see Fig. 2). In marked contrast, knowledge about the receptors involved in TRL interactions with the endothelium is in its infancy and as previously noted [26] virtually nothing is known about the specific receptor mechanisms mediating CMR actions on ECs. There is an indication that TRL isolates (consisting largely of VLDL) may bind to the VLDL receptor on ECs and VSMC [119], whereas RLP potentially interact with EC and VSMC LOX-1 [108,120]. RLP have been reported to increase expression of LOX-1 in an EC cell line (CRL-1730) and in this study Nox-dependent superoxide production as well as TNF-α and IL-1β synthesis induced by incubation with RLP was abrogated by treatment with a LOX-1 antibody [108]. Transfection with an antisense LOX-1 oligonucleotide also modulated RLP-induced inflammatory changes in this cell line, abrogating RLP-driven superoxide production, TNF-α and MCP-1 expression, and expression of VCAM-1 [120]. Expression of low density lipoprotein receptor-related protein 1 (LRP1) and sortilin-related receptor (LR11), two members of the LDL receptor family, were both increased in human aortic ECs following exposure to postprandial TRL comprising mainly VLDL and CM and this upregulation was suppressed in the presence of receptor associated protein-1 (RAP-1), an LRP1 and VLDL receptor antagonist [24]. Other investigations in aortic ECs have shown that incubation (3 h) with products of LPL-mediated TRL lipolysis promotes translocation of LRP1 (along with caveolin-1 and endothelial NO synthase (eNOS)) from membrane lipid rafts to other regions of the membrane and that this is regulated by increased ROS production [121]. To the authors’ knowledge there have been no detailed investigations of the role of the ApoB48 receptor in vascular cells and a recent report has indicated that this receptor is not expressed at the mRNA level in ECs, at least in those of fetal origin [122]. In addition, uptake of ApoB48-containing lipoproteins was not evident in HUVEC whereas macrophage and hepatocyte cell lines showed marked internalization of labeled lipoproteins [122]. The fact that artificial ApoE-containing CMR-like particles devoid of ApoB48 promote rapid changes in intracellular signaling and modify gene expression in ECs [100] provides further evidence that ApoB48 is unlikely to be a major receptor governing the actions of CMR on endothelium. Overall, these studies suggest that the LRP1, VLDL receptor and LOX-1 are potential candidates for mediating the receptor-dependent effects of heterogeneous populations of TRL on ECs but the receptors important for CMR interactions with ECs and VSMCs require further characterization.

4.4. Lipolysis-dependent interactions of TRL with endothelium

In the circulation, VLDL and CM TG are hydrolysed by an apoCII-dependent interaction with LPL, which is tethered to ECs of the capillary beds within adipose tissue, cardiac muscle and skeletal muscle by HSPGs and/or glycosyl phosphatidylinositol binding (see [74]). LPL activity releases free fatty acids (FFA) that are taken up by the proximal tissue where they can be esterified, oxidized to obtain energy, or used in synthetic pathways, whilst some FFA remain in the circulation bound to albumin.

Studies addressing the role of LPL in modulating the cellular actions of TRL have focused mainly on regulation of endothelial permeability, a parameter which is influenced by the integrity of endothelial adherens junctions through regulation of vascular
endothelial (VE)-cadherin phosphorylation. These junctions are dissociated by inflammatory EC activation, contributing to the transendothelial migration of monocytes during atherogenesis (see [123] for review). In keeping with studies demonstrating a lack of effect of ‘normal’ TRL on EC signaling (e.g. [24]) several investigations have reported that incubation of cultured human ECs with TRL fractions composed largely of VLDL and LDL remnants has negligible effects on endothelial monolayer permeability as assessed by measurements of transendothelial electrical resistance (e.g. [124]). Exposure of ECs to a combination of these TRL and recombinant LPL, however, resulted in increased permeability accompanied by rearrangement of the actin cytoskeleton, changes in cellular localization of junctional proteins and increased caspase-3 activity, consistent with pro-apoptotic activity [124]. Since only marginal changes in VE-cadherin localisation were evident in cells exposed to TRL and LPL, these effects most likely occur through mechanisms that are distinct, at least in part, from those used by other factors with known ability to enhance monolayer permeability (e.g. VEGF), but are thought to involve changes in intracellular ROS production [121]. Other studies have shown that the n-6 PUFA, linoleic acid, is a major product of TRL and LPL co-incubation, that a range of oxidized FFA are also generated, and that these fractions elicit largely pro-inflammatory effects on arterial ECs, including cytokine synthesis and increased adhesion molecule expression [125]. TRL lipolysis-stimulated activation of ECs is also characterized by increased ROS production, generated mainly through Nox-dependent mechanisms, and by enhanced permeability [121,125]. Thus, there is evidence that TRL metabolism by LPL generates lipid species which can co-operatively facilitate inflammatory activation of aortic endothelial cells in vitro, suggesting a mechanism through which TRL could influence the activation state of the endothelium to promote endothelial dysfunction. On the other hand, lipolytic processing of lipoproteins may also regulate peroxisome proliferator activated receptor (PPAR) activation through generation of PPAR ligands. Activation of PPARs (comprising α, β/δ and γ isoforms) is complex and serves a multitude of roles in metabolism and inflammation, but it is generally accepted that these nuclear receptors mediate largely anti-inflammatory protective responses in ECs. It has been reported that VLDL hydrolysis by LPL generates ligands for PPARα [126]. ApoCIII is an endogenous LPL inhibitor and in the presence of LPL, ApoCIII-depleted VLDL particles promoted transcriptional activation of PPARα to a greater extent than unmodified VLDL, confirming that this protein regulates access to lipoprotein-derived PPAR ligands [126]. Lipolysis of HDL also produces PPAR ligands, a lipolytic pathway which seems to result in preferential activation of PPARα and limits leukocyte interaction with ECs through inhibition of endothelial VCAM-1 expression [127]. Similarly, an anti-inflammatory role has been suggested in other studies where LPL treatment of VLDL and subsequent incubation with ECs inhibited TNF-α-induced VCAM-1 expression in a PPARα-dependent manner [128].

Overall, therefore, it would seem that LPL-mediated lipolysis of TRL may represent an endogenous mechanism for influencing local inflammatory of the endothelium. It is highly possible that FFA generated during hydrolysis of the different TRL present in the postprandial phase will differentially modify EC function depending upon their lipid composition (see below), but this has not yet been investigated simultaneously. Despite the fact that most studies have used large vessel ECs, the precise functional significance of this mechanism for large vessel endothelium remains to be determined since GPⅢbⅣⅡa, the EC transporter for LPL in vivo, has been reported to reside exclusively in capillary endothelium (see [74]). This suggests that microvascular ECs, particularly those in the heart, adipose tissue and skeletal muscle, are most likely to be affected by the high local concentrations of FFAGenerated by LPL-mediated TRL hydrolysis, whereas changes in large vessel ECs lacking LPL expression may be influenced more prominently through effects mediated by ‘whole’ particles. In addition, there is some evidence that rodent ECs exposed to hypercholesterolemic serum accumulate lipid and have been termed ‘EC-derived foam cells’ [94]. It has been suggested that ECs on the surface of atherosclerotic lesions show these phenotypic changes (see [129]) and that these large vessel ECs are also capable of LPL-mediated TRL lipolysis (see [130]). It is not known whether ECs associated with human atherosclerotic plaques show similar changes or whether this has consequences for the specific actions of postprandial TRL and products of their lipolysis on plaque-associated ECs.

4.5. Endothelial actions of TRL determined by fatty acid composition

There is a vast literature describing the direct actions of FFA on a broad range of vascular and non-vascular cell types in vitro, consideration of which is beyond the scope of this review. However, with respect to the vascular effects of TRL in the postprandial phase there is increasing evidence to support the hypothesis that these particles exert differential actions on the endothelium depending upon their fatty acid composition. As noted above, TRL isolated from the plasma following meals with high saturated fat content or from patients with hyperlipidaemias trigger largely pro-inflammatory effects on ECs in vitro, most likely through pathways involving enhanced ROS production. In contrast, there are some reports that TRL isolated from the plasma of individuals ingesting meals with high MUFA or PUFA contents can abrogate, at least to some extent, the actions of an overt inflammatory stimulus on ECs (see [26]). For example, postprandial TRL from MUFA- or PUFA-fed individuals have been reported to inhibit TNF-α-induced EC activation to a greater extent than those from the plasma of subjects consuming SFA [131]. Similarly, there is evidence that TRL from humans consuming a MUFA-rich meal directly increase NO synthesis by HUVEC [132], indicating a beneficial action on ECs. Other studies in HUVEC have shown that exposure to CMR-like particles containing n-6 PUFAs causes reduced NO formation (as assessed by measurements of cGMP formation), suggesting a detrimental effect on ECs [100]; these findings are consistent with the fact that n-6 PUFA-derived eicosanoids (e.g. prostaglandin E2 (PGE2) and leukotriene B4) can exert pro-inflammatory actions that would be expected to contribute to endothelial dysfunction [133]. Recent studies have provided additional evidence that the ratio of n-6 to n-3 PUFAs is an important determinant of atherosclerosis development, at least in rodent models [134]. This study made use of the transgenic fat-1 mouse model where fat-1 encodes an n-3 PUFA desaturase which converts n-6→n-3 PUFA; this results in a high n-3 content and a lower ratio of n-6/n-3 PUFA in cells and tissues (including aorta and monocytes) following ingestion of a diet rich in n-6 but devoid of n-3 PUFA [134]. Ex vivo culture of ApoE−/− fat-1 aortas showed markedly less production of inflammatory cytokines (e.g. IL-6, MCP-1) and reduced expression of genes associated with inflammation (e.g. ICAM-1, COX-2) compared to aortas from ApoE−/− animals, together with lower circulating cytokines and suppressed formation of atherosclerotic lesions [134]. While these studies did not specifically investigate EC function and the relevance for humans awaits clarification, the results strongly suggest that decreasing the n-6/n-3 ratio is likely to have beneficial anti-inflammatory actions within the vasculature.

There is currently very little information available regarding the endothelial actions of postprandial TRL of defined fatty acid composition and particularly those containing n-3 PUFA. Highly buoyant TRL (S > 400) from the postprandial plasma of subjects ingesting a meal supplemented with fish oil appeared to increase eNOS mRNA expression in HUVEC, although this was not confirmed at the protein level [135]. These changes in eNOS mRNA
were accompanied by reduced expression of Nox4 mRNA but this is unlikely to represent a ‘fish-oil’-specific effect since control TRL isolated following a typical placebo meal had a similar inhibitory effect on Nox4 mRNA expression [135]. The significance of changes in Nox4 expression/activity for EC function is still debated so it is unclear whether altered expression of this Nox isoform by TRL would translate to a beneficial or detrimental effect on EC function.

Although there is emerging evidence that the overall oxidation state of CMR strongly influences their uptake and effects on macrophage cytokine production (see below), there is currently no appreciation of how the oxidation state of CMR influences their interactions with ECs. Since overall oxidation status will be strongly influenced by FA composition this has direct consequences for understanding how different dietary lipids modify vascular function in the postprandial period and awaits clarification. The potential role of oxPL has been discussed above and the possibility that these components exert anti- and pro-inflammatory effects on ECs highlighted. There is also evidence that specific oxidation products of n-3 PUFA may exert beneficial effects on the vasculature; these fatty acids are highly unsaturated and readily undergo oxidation, a modification that may well be required for their anti-inflammatory activity. For example, exposure of HUVEC to oxDHA and ox-eicosapentaenoic acid (EPA) reduces cytokine-stimulated NF-κB activation, MCP-1 expression and IL-8 release, effects which were not observed following treatment with unmodified DHA or EPA [136]. Moreover, these actions seem to depend upon PPARα since they were abolished in aortic ECs isolated from PPARα-deficient mice [136].

Cyclopentanone metabolites produced by oxidation of both DHA and EPA also appear to protect cultured ECs from the pro-inflammatory, pro-atherogenic actions of organic pollutants [137]. In this study incubation with oxDHA abrogated pollutant-induced superoxide production, NF-κB activation and MCP-1 expression in HUVEC and direct exposure to DHA-derived cyclopentanones increased DNA binding of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and downstream expression of the Nrf2-dependent gene NAD(P)H quinone oxidoreductase [137]. Interestingly, direct application of EPA at concentrations above 25 μM to bovine aortic ECs activates AMP kinase (AMPK), phosphorylates eNOS and enhances NO production which appears to equate to improved vascular function in rodent aortas ex vivo [138]. Given the documented anti-inflammatory roles attributed to AMPK, its activation in ECs following treatment with EPA is consistent with the hypothesis that n-3 PUFA are cytoprotective. The endothelial actions of TRL specifically enriched in n-3 PUFA remain unexplored, but the findings discussed above are in keeping with our recent observations that CMR-like particles containing fish-oil TG exert anti-inflammatory actions on human ECs through mechanisms that include Nrf2-dependent HO-1 induction (Latham et al., unpublished). Thus, the vascular actions of TRL containing n-3 PUFA may oppose or limit those of TRL enriched in SFA or n-6 PUFA – improved mechanistic insight into these interactions is therefore key to understanding how TRL influence EC function in the postprandial phase. Interestingly, several polyphenolic- and thiol-containing phytochemicals capable of redox cycling have also been identified as Nrf2 agonists, providing a mechanism by which foods rich in these compounds may protect against disease-associated oxidant stress (see [139]). Whether the vascular actions of these beneficial foodstuffs are additive or synergistic with the potentially protective actions of n-3 PUFA-containing CMR remains to be determined.

While overproduction of ROS accompanies endothelial dysfunction and seems to contribute to the inflammatory effects of TRL (see above), low levels of intracellular ROS (O₂⁻ and H₂O₂) have documented roles as second messengers, contributing to normal cell signaling and function as well as to the pathophysiological signaling associated with inflammatory challenge. In this respect it is notable that recent studies have emphasized the potential importance of Nox-4 in conferring endothelial cytoprotection under certain conditions [140]. These authors showed that Nox-4-deficient lung microvascular ECs were characterized by reduced expression of Nrf2 and that loss of Nox-4 was coupled to reduced eNOS and HO-1 expression, and increased EC apoptosis. Apoptosis associated with Nox-4 deletion was reversed by exogenous carbon monoxide, a product of HO-1 activity [140]. Thus, in murine ECs at least, Nox-4-dependent regulation of HO-1 contributes to cytoprotection. Whether this pathway is important for mediating the vascular actions of TRL containing n-3 PUFA is not known, but it is conceivable that these particles could use this mechanism to regulate expression of the anti-oxidant anti-inflammatory HO-1. The implication that the fatty acid composition of TRL (including the CMR component) could directly influence the balance of EC signaling and function to differentially pre-dispose to vascular damage (pro-inflammatory) or protection (anti-inflammatory, anti-oxidant) now needs to be investigated fully using physiological TRL isolated from humans as well as CMR-like particles prepared using natural dietary oils.

4.6. Summary

In summary, the literature to date supports the hypothesis that in the postprandial phase the lipoprotein carriers of dietary fats directly influence EC signaling and function and there is emerging evidence that the overall outcomes of these interactions in terms of vascular homeostasis are dependent upon the fatty acid composition and oxidative state of the particles. The actions of these lipoproteins on large vessel and potentially microvascular ECs are likely to be mediated by at least 3 inter-linked mechanisms: (i) direct interaction of the particles with cell surface receptor(s) and subsequent engagement of downstream signaling; (ii) LPL-mediated lipolysis with release of FFA and changes in signaling mediated through extracellular FFA receptors and/or intracellular actions; and (iii) receptor-(in)dependent pinocytosis followed by intracellular lipolysis and consequent changes in cell function mediated through FFA products.

Inflammation is now taking center stage as a major driver of atherogenic changes in the vasculature. Overall, it is evident that exogenous lipoproteins and their components exert direct and/or indirect effects on ECs to modify pro- and anti-inflammatory gene expression. These changes most likely depend upon the integrity of the endothelial glyocalyx and are driven by acute alterations in intracellular signaling events with current evidence suggesting prominent roles for pathways involving p38MAPK, MEK-ERK, and NF-κB and emerging involvement of Akt and potentially AMPK (see Fig. 2). There is an indication that Nox enzymes are likely to be important determinants of the signaling pathways engaged and of the consequent changes in gene expression and EC function. The differential roles of individual Nox isoforms as mediators of detrimental and protective EC responses to CMR have not been explored to date but could shed light on how intracellular oxidant status contributes to the vascular actions of TRL. Collectively, the studies highlighted reinforce the importance of dietary-derived lipoproteins as modulators of EC signaling and function during the postprandial phase. Fatty acid composition is likely to be an important determinant of their cellular actions at the molecular level and detailed investigations testing this hypothesis are now warranted.

5. Postprandial lipoproteins and molecular regulation of monocyte/macrophage function

Monocytes are the main inflammatory cell type which invade the artery wall in atherosclerosis, and their subsequent differentiation into macrophages and the formation of foam cells is
implicated all stages of lesion development [141,142]. Factors which influence the behavior of the monocyte/macrophage, therefore, play a crucial role in the progression of the disease.

5.1. Monocyte activation

Monocytes are said to be activated after exposure to inflammatory agents such as cytokines and chemokines causes changes in their morphology and behavior. Activated cells are characterized by raised expression of CD11b, which enhances their tendency to adhere to the endothelium, together with increased production of ROS and of pro-inflammatory cytokines and chemokines such as IL-8 and MCP-1. These effects, together with concomitant changes in ECs which lead to raised expression of adhesion molecules such as ICAM-1, VCAM-1 and selectins, promotes monocyte adhesion to the endothelium and invasion of the artery wall [21,141,142].

Postprandial TRL from healthy subjects, model CMR-like particles, and lipolysis products from human VLDL isolated after a fat meal have been shown to cause lipid accumulation in primary human monocytes or in the human monocyte cell line, THP-1, in experiments in vitro [66,143–145]. Moreover, in studies in vivo, monocytes isolated from healthy human volunteers after a high fat meal have been found to be enriched in meal-derived fatty acids [146], to contain lipid droplets not seen in cells from fasting blood [147], and to accumulate lipids in a time-dependent manner which coincides with the postprandial increase in apoB48-containing TRL [143]. In addition, apoB binding to monocytes in the postprandial phase has been reported by Alipour et al. [146]. Monocytes, therefore, become enriched in intracellular lipids postprandially by uptake from TRL.

The mechanisms by which TRL are taken up by monocytes have not been studied extensively; however, recent work has begun to shed light on the receptor-mediated pathways which may be involved. Muriana and colleagues [143,144] have shown that the expression of mRNA for the apoB48 receptor (apoB48R) is up-regulated in monocytes in parallel with the increase in postprandial apoB48-containing TRL in healthy men following fat intake. This effect was also reproduced in vitro with THP-1 monocytes, and the amount of lipid accumulated in response to the lipoproteins was markedly reduced in the presence of siRNA targeting the apoB48R. Further experiments showed that PPARα and PPARγ antagonists as well as siRNA targeted to these transcription factors suppressed the effects of TRL on apoB48 mRNA expression, while PPAR agonists had the opposite effect; activation of retinoid X receptor (RXR), a transcriptional partner of PPARs, also potentiated the effects of TRL. These studies provide evidence to suggest that the transcriptional regulation of apoB48R expression by TRL in monocytes is mediated via PPARα- and PPARγ-dependent pathways [144]. In a study with primary human monocytes, however, Gower et al. [147] found evidence indicating a role for LRPI in the uptake of TRL by primary human monocytes, since internalization was inhibited by its antagonist, Rap and the presence of TRL inhibited LRPI antibody binding. Thus, current evidence suggests that apoB48R and LRPI participate in TRL uptake by monocytes, but as yet only a few studies have investigated the process and it seems likely that, as is the case with macrophages (see below), other receptors are also involved. The LDL receptor (LDLR), for example, is a potential candidate as, like LRPI, it recognizes apoE, one of the main apolipoproteins found on CMR [16,37].

Postprandial lipemia and TRL have been linked to an increase in a number of markers of monocyte activation, including CD11 and CD14 expression, adhesion to VCAM, chemokinesis to MCP-1 and the production of superoxide, ROS and IL-8. Monocytes isolated from healthy volunteers after oral fat loading and from patients with diabetes have been reported to show increased expression of CD11b, CD11c and/or CD14 [146,147–149] and these changes were correlated with changes in blood TG levels. In addition, native postprandial TRL and model TRL-like particles have been demonstrated to up-regulate CD11b or CD11c expression in primary human monocytes in vitro [146,147]. Gower et al. [147] measured monocyte adhesion to recombinant VCAM-1 in diluted whole blood under defined shear stress, and found that there was an increase when cells were isolated from healthy individuals after a fat meal which was linked to the rise in their expression of CD11c and also to blood TG concentrations. RLP prepared from TRL isolated from hypertriglyceridemic patients after a fat-containing breakfast have also been shown to cause increased adhesion of human U937 monocytes to HUVEC and this was related to increased expression of integrins, RHoA activation mediated by protein kinase C (PKC) signaling and focal adhesion kinase (FAK) activation [150]. Studies by Sacks and colleagues have demonstrated that the apoCIII content of apoB-containing lipoproteins, including TRL, may play a role in their induction of monocyte adhesion to the endothelium [111,151–153]. TRL enriched in ApoCIII were found to promote adhesion to ECs, and this appeared to be due to raised VCAM-1 expression by ECs together with upregulation of integrin expression in the monocytes [111,151,152]. Related experiments also suggest that effects of ApoCIII are associated with PKC signaling and activation of TLR-2 and NF-κB [152,153].

Other in vitro studies with THP-1 monocytes have demonstrated a large, rapid and prolonged rise in ROS production in the presence of model CMR-like particles together with an increase in IL-8 secretion [66], and an increase in superoxide production by blood mononuclear cells in patients with hypertriglyceridemia has also been reported [154]. Experiments using pharmacological inhibitors suggested that the rise in ROS production is mediated by NF-κB, and this is consistent with previous work showing that intake of butter or walnut oil fat activates NF-κB in peripheral blood mononuclear cells from healthy volunteers [155]. No evidence was found, however, for the involvement of ERK1/2, Nox isoforms or xanthine oxidase [66]. Surprisingly, this study also found that CMR-like particles markedly decreased the secretion of MCP-1 by monocytes, although it has been reported previously that reduced ROS formation results in suppression of NF-κB-dependent MCP-1 secretion in monocytes [156] and that production of MCP-1 in VSMCs is increased both by CMR and ROS [82]. Further investigation, however, showed that reduced levels of monocyte-derived MCP-1 in the culture medium of monocytes caused by exposure to CMR-like particles promoted cell migration towards a higher concentration of MCP-1, and that this effect was reversed by replacement of MCP-1 in the medium after the incubation with the lipoproteins. It was proposed, therefore, that the down-regulation of MCP-1 secretion in monocytes induced by CMR leads to an increase in the chemoattractant gradient across the endothelium and thus has pro-migratory effects on circulating monocytes [66]. Monocyte migration may also be enhanced by the rise in IL-8 secretion promoted by CMR [66], as IL-8 has been shown to cause monocyte activation during their adhesion to the endothelium [157].

The effects of the oxidative state of CMR on the activation of monocytes were investigated by Armengol Lopez et al. [158] using CMR-like particles and THP-1 monocytes. Interestingly, oxidation of the particles had no effect on their uptake by the cells or their induction of lipid accumulation, promotion of ROS production or suppression of MCP-1 secretion. However, when the lipoproteins were protected from oxidation by incorporation of antioxidant into the particles, no lipid accumulation could be detected and the production of ROS and MCP-1 was unaffected. Thus, it seems that the activation of peripheral blood human monocytes by TRL may be down-regulated by dietary antioxidants. This adds to the considerable experimental evidence suggesting that these micronutrients may help to retard the early development of atherosclerotic lesions, although attempts to demonstrate anti-atherogenic effects
in large trials with healthy subjects in vivo have so far proved unsuccessful [159].

It is clear from the studies discussed above that TRL are taken up by monocytes causing lipid to accumulate intracellularly, and that they also promote an increase in the inflammatory state of the cells which enhances their propensity to adhere to the endothelium and enter the artery wall. The mechanisms involved in these processes are now starting to emerge. Current ideas are summarized in Fig. 3.

5.2. Macrophage foam cell formation

The fatty streak, consisting mainly of lipid engorged macrophage foam cells, is the first visible lesion in atherosclerosis. The role of LDL, particularly after oxidative modification, in the induction of foam cell formation is well established [4–6], but there is now a great deal of evidence to implicate TRL in the process. The uptake of TRL by macrophages and subsequent induction of foam cell formation has been demonstrated unequivocally in experiments ex vivo and in vitro using TRL (isolated postprandially or from patients with hypertriglyceridemia or diabetes), CMR (prepared from human or animal CM) and CMR-like particles together with human and animal primary macrophages and macrophage cell lines [37,65,130,160–162].

The amount of lipid accumulated during foam cell formation clearly depends on the balance between the amount taken up and the amount effluxed from the cells. However, although many studies have investigated TRL uptake by macrophages, much less attention has been paid to the subsequent efflux following lipid uptake. In vitro studies in THP-1 macrophages have shown that the expression of mRNA for ATP binding cassette transporter A1 (ABCA1), a protein involved in cholesterol efflux, is decreased by CMR-like particles [163]. In addition, the cholesterol taken up was found to be resistant to efflux in the presence of a potent cholesterol acceptor (apoA-I-phosphatidylcholine vesicles), and the mass of TG in the cells over a period of 48 h was not decreased in any conditions [164]. Evaluation of the subcellular localization of the lipid suggested that the observed resistance to efflux may be due to its sequestration in lysosomes [163], as has been reported for oxLDL [165,166].

It has been known for many years that the type of fat in the diet influences atherosclerosis development. Higher intake of SFA is associated with increased risk, while increased consumption of MUFA or PUFA has a protective effect [167,168]. SFA is found mainly in animal fat, while MUFA and PUFA of the n-6 series are found in plant oils and PUFA of the n-3 series in fish oil. Since the fatty acid composition of TRL reflects that of the diet, it is possible that the different dietary fats they carry may modulate their effects on macrophage foam cell formation. Using CMR-like particles enriched in TG containing SFA, MUFA or n-6 or n-3 PUFA and THP-1 macrophages, De Pascale et al. [169] found that lipid accumulation in the cells was increased when the particles were enriched in SFA as compared to those high in MUFA or n-6 or n-3 PUFA, mainly because of a rise in TG concentrations. Moreover, this effect was found to be due both to a higher rate of uptake and a lower rate of efflux of the SFA-containing lipoproteins [169,170]. Decreased lipid accumulation in THP-1 macrophages in response to TRL enriched in n-3 PUFA has also been demonstrated in experiments with mouse VLDL isolated after feeding the animals a control diet or a diet high in eicosapentaenoic acid [171].

Many studies have demonstrated that, in marked contrast to LDL, TRL are able to induce foam cell formation without prior oxidation (for reviews see [37,65,130]). However, the oxidative state of TRL may be modulated by dietary oxidized lipids or antioxidant micronutrients such as vitamins and carotenoids carried in CM and CMR and by oxidation when trapped in the artery wall, as occurs with LDL [37,76,77]. It is important, therefore, to understand how the oxidation of TRL influences their induction of foam cell formation. Studies using CMR-like particles with THP-1 macrophages or human monocyte-derived macrophages (HMDM) have demonstrated that the effects of oxidation of CMR as compared to LDL are strikingly different. Thus, the amount of lipid accumulated by macrophages is inversely related to the oxidative state of CMR, so that particles protected from oxidation by the incorporation of antioxidants which may be present as micronutrients in the diet, such as the tomato pigment lycopene or the antioxidant drug, probucol, cause approximately twice as much lipid to be accumulated as the unprotected lipoproteins, while oxidation of the particles has the opposite effect [37,162,172–174]. Furthermore, this has been shown to be due to changes in the rate of uptake of the particles [162] and increased intracellular synthesis of TG, but not cholesteryl ester [162,172]. On the other hand, in similar experiments in which probucol or vitamin E were incorporated into

![Fig. 3. Monocyte activation by TRL.](image-url)
modified LDL, the amount of lipid accumulation induced in macrophages was reduced [175,176]. The inverse relationship between CMR oxidative state and macrophage foam cell formation contrasts with that observed with primary human monocytes, where incorporation of antioxidant into the particles had the expected result of reducing their induction of cell lipid content [66]. Thus, it is clear that TRL have markedly different effects before and after differentiation of monocytes into macrophages, emphasizing the importance of studying the processes in both cell types. Interestingly, probucol has the effect of promoting atherosclerosis in apoE or LDL receptor deficient mice, which have markedly elevated TRL levels [177]. In conditions where the clearance of TRL is delayed, therefore, dietary antioxidants may promote, rather than retard macrophage foam cell formation, and this may provide part of the explanation for the puzzling discrepancy between the extensive evidence from experimental studies suggesting that dietary antioxidants are anti-atherogenic, and the failure of large scale intervention studies to demonstrate any protective effects. [159].

It has been shown in extensive studies that the mechanisms of uptake of TRL by macrophages involve multiple receptor-mediated pathways, and that there may also be selective uptake of cholesterol ester via scavenger receptor-B1 (SR-B1), non-receptor-mediated uptake such as extracellular lipolysis of TG followed by internalization of the fatty acids released, and phagocytosis [37,65,162,178–180]. Uptake mechanisms are summarized in Fig. 4. Macrophage uptake of CMR has been found to be considerably decreased in the absence of apoE, and the LDLR was one of the receptors implicated initially [37,162]. However, studies with THP-1 macrophages have shown that expression of LDLR mRNA is down-regulated by CMR-like particles [162,163], suggesting that delivery cholesterol to macrophages by CMR suppresses receptor activity, as is well known to occur with LDL [6]. It is is also clear that TRL can still be taken up by macrophages, although at a lower rate, when the LDLR is absent or inhibited [37,162]. The second apoE-dependent receptor shown to play a part in the uptake of CMR is LR1. Inhibition of the receptor by ligands including RAP and lactoferlin has been found to reduce macrophage CMR uptake by 60–90% [162,181], and the expression of LR1 mRNA in THP-1 macrophages has also been shown to be increased by CMR-like particles [162,163]. In addition, Takahashi and colleagues have demonstrated that a third apoE-dependent receptor, the VLDL receptor (VLDLR), is expressed in human macrophages, and that the binding of TRL to this receptor is accelerated by apoE and LPL secreted by the cells [182,183]. It seems likely, therefore, that the VLDL component of TRL enters by this route.

Human macrophage apoB48R, which binds to apoB48 and the equivalent domain on apoB100 and was first identified and cloned by Brown et al. [184], is also thought to contribute to TRL uptake. Lipid accumulation in THP-1 macrophages exposed to postprandial TRL or RLP has been found to be markedly decreased by siRNA targeted to apoB48R [144,185]. However, in macrophages treated with postprandial TRL, expression of mRNA for the receptor was down-regulated, in contrast to the opposite effect of up-regulation observed in THP-1 monocytes [144]. In experiments with THP-1 cells, Bermudez et al. [144] also found that, in the presence of TRL, activation of PPARα, PPARγ and RXR had opposite effects on apoB48R mRNA abundance to that observed in monocytes, so that expression was suppressed by agonists, and enhanced by antagonists and siRNA targeted to PPARs. In agreement with this, another study has reported that PPARα and γ activators decrease the expression of mRNA and protein for apoB48R in THP-1 macrophages, and also lower the amount of TG accumulated by HMDDM after incubation with trypsinized VLDL [186]. Current evidence suggests, therefore, that PPAR-dependent pathways regulate apoB48R expression in monocytes and macrophages in opposite ways, so that apoB48R transcription is increased by TRL in a positive feedback loop in monocytes, as happens with scavenger receptors in macrophages treated with oxLDL [187], while a negative-feedback process which suppresses transcription similar to that seen with the LDLR and native LDL [4–6] operates in macrophages. These differences again highlight the differential effects of TRL in monocytes versus macrophages.

Scavenger receptors such as scavenger receptor A (SR-A) and CD36 are known to play an important part in the induction of foam cell formation by modified LDL [188]. However, there is scant evidence to suggest they play anything but a minor role in the uptake of TRL by macrophages, although it has been reported that the uptake of modified LDL by HMDDM is increased after their exposure to human postprandial TRL, suggesting that scavenger receptor activity is increased [189]. Studies by Bejta et al. [162] using pharmacological inhibitors of potential receptors for CMR concluded that CD36 may be responsible for the uptake of a small proportion of the particles by THP-1 macrophages, but that SR-A is not involved. However, a possible role for SR-B1 in the selective uptake of cholesterol ester from CMR without internalization of the whole particle, analogous to the mechanism known to mediate the uptake of cholesterol ester from HDL via this receptor in the liver [190], has been identified by Napolitano and Bravo [178]. Using radiolabeled CMR, internalization of cholesterol ester, but not TG, by HMDDM was found to be inhibited by an antibody to SR-B1, although the two lipids were carried in the same particles.

Since macrophages secrete a number of lipases, including LPL and phospholipase A2, extracellular lipolysis of TRL TG, followed by uptake of the fatty acids released and resynthesis of TG within the cells, is another possible mechanism by which the lipoproteins may promote lipid accumulation in macrophages. Evidence to support this has come from numerous studies showing that the increase in cell-associated TG in THP-1 macrophages or HMDDM induced by TRL isolated postprandially from normolipidemic subjects, CMR-like particles or VLDL isolated from hyperlipidemic subjects or mice was attenuated by inhibitors of LPL and/or phospholipase A2 [171,180,191–193]. Phagocytosis has been reported to be important in the uptake of CMR by rabbit alveolar
macrophages [194,195], but other studies suggest it is not involved or has only a minor role in the internalization of CMR by THP-1 macrophages and HMDM [162,180].

Although oxidative modification of CMR has profound effects on their induction of lipid accumulation in macrophages, Bejta et al. [162] found that the uptake of CMR, oxCMR and CMR containing the antioxidant probucol by THP-1 macrophages was mediated mainly by the LDLR and LRP1, and although lipid accumulation was inversely related to the oxidative state, this was not accompa- nied by any major change in the route of uptake such as that which occurs on oxidation of LDL [6]. It has been suggested, on the other hand, that the presence of oxysterols in CMR-like particles interferes with the mechanisms of uptake, although the nature of these changes has not yet been defined [189].

5.3. Macrophage inflammatory processes

Macrophages are known to contribute to the inflammatory re- sponse to modified lipoproteins and oxidative stress in the artery wall in a number of ways: by producing ROS which cause LDL oxida- tion; by secreting inflammatory chemokines and cytokines such as TNF-α, IL-1β and MCP-1; and via the production IL-6 which stimulates the release of C-reactive protein (CRP) from the liver and adipose tissue [4,196].

The pro-inflammatory effects of oxLDL on macrophages are known to play a major part in its promotion of atherosclerosis development [5,6,197,198]. Much less information is available, however, about the modulation of macrophage inflammatory pro- cesses by TRL, although some pieces of the puzzle are now starting to emerge. Recent work, detailed below, has provided evidence to suggest that TRL influence the secretion of pro-inflammatory che- mokines and cytokines by macrophages via cell signaling pathways mediated by NF-κB and ERK1/2 and possibly also p38 MAPK and JNK, and that these effects are modulated by the receptor-mediated route by which they enter the cells as well as by the type of dietary fat they carry and their oxidative state.

A number of studies have investigated the effects of TRL on che- mokine/cytokine secretion by macrophages, but the results appear to depend on the type of lipoprotein assessed, so that in general, CMR seem to suppress and VLDL enhance secretion, with TRL containing mixed classes showing a pattern somewhere in between. The secretion of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β and MCP-1 has consistently been found to be decreased by CMR-like particles in THP-1 macrophages and/or in HMDM, with the effect on TNF-α and MCP-1 production being particularly strong (>90% or more), while that of the anti-inflammatory transforming growth factor (TGF)-β is unaffected; moreover, the expression of mRNA for the cytokines showed a similar pattern [170,174]. Similar effects on MCP-1 and IL-6 secretion were observed when TRL isolated postprandially from normolipidemic volunteers were used, but in this case TNF-α and IL-1β secretion was increased rather than decreased [160]. VLDL prepared from humans or mice, on the other hand, has been reported to enhance the secretion and expression of mRNA for TNF-α, IL-1β, MCP-1 and macrophage inflammatory protein (MIP)-1α, as well as the expression of both protein and mRNA for ICAM-1 and MMP3 in HMDM and mouse peritoneal macrophages [192,199–201]. Persson et al. [201], however, found that after lipid loading with VLDL (origin not given), the secretion of TNF-α, IL-6 and IL-8 by HMDM was decreased.

The effects of the type of fat or lipophilic micronutrients carried in TRL on their modulation of macrophage cytokine secretion has been addressed in only a few studies, but the results so far suggest that the particles may be less inflammatory when they are enriched in n-3 PUFAs as compared to other types of fat. Preliminary work in our laboratory has demonstrated that CMR-like particles high in TG containing the n-3 PUFAs, DHA, suppress TNF-α and MCP-1 secretion more strongly than those containing SFA (Paola Di Maggio, CPD Wheeler-Jones and Kathleen M Botham, unpublished results). In addition, the up-regulatory effects of mouse VLDL on the expression of mRNA for IL-1β, TNF-α and MCP-1 in THP-1 macrophages have also been reported to be attenuated when VLDL from mice fed a diet enriched in the n-3 PUFA, EPA, as compared to a control diet was used [171]. Since oleanolic acid, a lipophilic micronutrient found in higher amounts in blended olive oil [160], has been found to suppress the secretion of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 by peripheral blood mononuclear cells [202], its potential effects on postprandial inflammation have been investigated ex vivo using TRL isolated from healthy volunteers shortly after a test meal containing virgin olive oil (V), pomace oil (P), or pomace oil supplemented with oleanolic acid (POA) [160]. The re- sults showed that the increase in the secretion of TNF-α and IL-1β by THP-1 macrophages caused by POA TRL was lower than that caused by V and P TRL, and that POA TRL had a stronger down-reg- ulatory effect on COX-2 mRNA and protein expression. Thus, the micronutrients of TRL may also be important modulators of postprandial inflammation, but work in this area is still at a very early stage.

Despite the wealth of evidence indicating that oxidative modi- fication of LDL enhances its inflammatory effects [197], little attention has been paid to the effects of oxidation of TRL on macrophage inflammation. One study with CMR-like particles, however, has provided some important insights. Graham et al. [174] showed that oxidation of the particles had little effect on their suppression of pro-inflammatory cytokine secretion or mRNA expression by THP-1 macrophages or HMDM, but that the effects were reduced or completely abolished when they were protected from oxidation by incorporation of probucol. Using siRNA targeted to the LDLR or LRP1, the two receptors responsible for >90% of the uptake of CMR-like particles by macrophages [162], the authors went on to show that when LRP1 expression was blocked in THP-1 macrophages, normal and probucol-protected CMR-like particles had a similar inhibitory effect on cytokine secretion, but when LDLR expression was knocked down, the result was strikingly different, with neither type of particles now having any significant effect. These experi- ments demonstrate that uptake of CMR via LRP1 does not trigger the suppression of macrophage cytokine secretion, but that internalization via the LDLR is required for this effect. The findings also suggest that the LDLR plays a relatively small part in the uptake of antioxidant protected particles by macrophages in normal circumstances, thus they have little effect on cytokine secretion, but has a more significant role in the uptake of normal particles, which is sufficient for them to suppress production. When the expression of LRP1 is down-regulated, however, a greater proportion of both types of particle enters via the LDLR, and thus both have an inhibitory effect (see Fig. 5). Thus, although uptake via the LDLR is quantita- tively less important for CMR in all oxidative states [162], it appears to be crucial for the modulation of inflammatory cytokine secretion by the particles.

The studies focusing on modulation of macrophage cytokine production by TRL discussed above have also uncovered some tantalyzing pieces of evidence concerning the signal transduction pathways involved. Since the expression of cytokines such as TNF-α, IL-6 and MCP-1 is transcriptionally regulated by NF-κB, De Pascale et al. [170] investigated the effects of CMR on its activa- tion in experiments with CMR-like particles and THP-1 macrophages. NF-κB transcriptional activity was decreased by the particles, and mRNA expression for COX-2, an NF-κB target gene, was also reduced, suggesting that CMR influence macrophage cyto- kine secretion by modulation of NF-κB activity. In contrast to these findings, Okumura et al. [203] reported that rat CMR promote IL-1β secretion and mRNA expression and that this was related to
Little is known about the mechanisms by which the fatty acid content of VLDL influences macrophage inflammation. However, based on results showing the increase in pro-inflammatory cytokine secretion caused by mouse VLDL in THP-1 macrophages is suppressed both by VLDL enriched in n-3 PUFA and by inhibition of LPL, it has been proposed that dietary n-3 PUFA may decrease inflammation by reducing the susceptibility of VLDL to lipolysis by LPL secreted by the cells [171].

Macrophages have a dual role in inflammation, as during infection inflammation is a protective mechanism against pathogens, but when the threat has been dealt with the deleterious effects on the tissues must be suppressed to allow healing. Thus, the cells may reversibly adopt a pro- or anti-inflammatory phenotype in response to different signals from the microenvironment [209,210]. Classically activated or type 1 (M1) macrophages respond to lipopolysaccharide and cytokines such as interferon (IFN)-γ to become pro-inflammatory, while alternatively activated (M2) macrophages are polarized towards anti-inflammatory phenotypes by cytokines such as IL-4, IL-13 and IL-10 [209,210]. In addition, another phenotype, designated Mox, has recently been identified. This state is promoted by oxidative stress and is characterized by elevated expression of antioxidant enzymes such as HO-1 [211]. Since it is clear that TRL influence macrophage inflammation, the question arises of whether they are able to switch macrophages from one phenotype to another. This idea is supported by a study by Baitsch et al. [212], who showed that apoE causes macrophage polarization towards the anti-inflammatory M2 state when the VLDLR is over-expressed in the murine macrophage cell line RAW264.7, and that VLDL deficient mouse bone marrow macrophages displayed an enhanced inflammatory phenotype. Since VLDL is thought to be taken up by macrophages mainly by the VLDLR, these findings suggest that TRL may play a role in modulating macrophage phenotype. The effects of CMR in suppressing pro-inflammatory cytokine secretion and NF-κB activity [170,174] suggest that they may also drive macrophages away from type M1 and towards alternatively activated phenotypes. No studies have been published on this as yet, but recent preliminary work in our laboratory with CMR-like particles and THP-1 macrophages has indicated that lipoproteins down-regulate M1 markers, while markers of alternatively activated phenotypes are up-regulated. Furthermore, these changes appear to be modulated by both the fatty acid composition and the oxidative state of the lipoproteins (Di Maggio P, Wheeler-Jones CPD, Botham KM, unpublished results). Although at an early stage, these findings suggest that CMR influence macrophage polarization by promoting a switch from the M1 to alternative phenotypes, leading to suppression of inflammation and enhanced expression of cytoprotective genes. This is a promising area for future investigations which has the potential to lead to important advances in the understanding of the part played by TRL in postprandial inflammation and how it may be manipulated by dietary changes or pharmaceutical intervention.

6. Summary and future directions

Growing focus on the relationship between diet and health in the developed world, particularly with regard to fat intake, emphasizes the importance of understanding the association between postprandial hyperlipidemia and heart disease, given that exposure of the vasculature to TRL represents an immediate and direct link between the lipid content of a meal and vascular function. Thirty years after the initial hypothesis put forward by Zilversmit [28], the current consensus view is that non fasting TRL levels are a significant risk factor for atherosclerosis development [12,29–33], and that direct interactions with circulating monocytes
and cells of the vasculature may play an important role in their atherogenic effects. [12,14,17,37,49].

The concept of postprandial inflammation is gaining momentum as a mechanism that may help to explain the potentially deleterious effects of remnant accumulation on vascular homeostasis. It is also recognized that these actions are likely to be exacerbated by existing vascular dysfunction associated with disease, obesity or old age. However, since postprandial inflammation is known to occur in healthy individuals after a high-fat meal [213,214], it could also represent a physiological response to dietary fat that does not, in itself, precipitate vascular dysfunction. In this respect there is good evidence to indicate that the atherogenicity of TRL is modulated by the dietary lipids they carry, including the degree of saturation or oxidation of the fats and possibly also by lipophilic micronutrients. This research area is still in its infancy but strongly suggests that the type of fat consumed in the diet will directly influence the extent and duration of vascular inflammation in the postprandial phase in both health and disease. This emphasizes the potential therapeutic importance of different dietary fats as regulators of vascular function.

TRL have been shown to be taken up by monocytes via receptor-mediated mechanisms, causing their activation and promoting their adhesion to the endothelium and invasion of the artery wall; to directly influence EC function and signaling; to contribute to macrophage foam cell formation after uptake by multiple receptor and non-receptor mediated routes and to influence macrophage inflammation by modulating cytokine secretion and possibly cell phenotype. There are also a few studies using rodent cells which suggest that VSMC function is also modulated by TRL but this work is still in an early stage. Studies have shown that the complex particles most likely target multiple signaling pathways in all these cell types to influence vascular homeostasis, including NF-κB, p38 MAPK, ERK1/2, Akt and AMPK. Furthermore, the combined effect of circulating TRL on these pathways appears to be dictated to a large extent by the lipid composition of the particles, and thus of the diet, and this determines whether the outcome of their cellular actions during the postprandial phase is largely beneficial (e.g. a diet high in n-3 PUFA) or potentially detrimental (e.g. a diet high in SFA and/or a low n-3: n-6 ratio) to vascular health. In addition, there is some limited evidence to suggest that lipophilic micronutrients may modulate the atherogenicity of TRL and this is a promising area for future studies.

Oxidative modification of TRL has been shown to alter the effects of the particles on monocyte activation, and on EC and macrophage function, often in ways which differ markedly from the effects of oxLDL, and this aspect needs further exploration. In addition to oxidation, however, there are other modifications that could influence the atherogenicity of TRL through modification of their capacity to promote inflammatory activation of ECs and or monocytes/macrophages. These include nitration, glycation and acetylation, all of which would be predicted to enhance the atherogenic actions of TRL; these are unexplored areas which deserve attention.

Although the molecular mechanisms by which TRL influence vascular function are now starting to be elucidated, further studies are clearly required and will, no doubt, bring clarity to these processes and provide novel information about how the biochemical heterogeneity of TRL present in the circulation in the postprandial phase facilitates or limits vascular cell function/dysfunction.

Acknowledgements

The authors thank the British Heart Foundation for financial support. Current studies on the vascular actions of dietary lipoproteins are funded by the BBSRC Diet and Health Research Industry Club (BB/1005862/1).

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