Modulation of the effects of chylomicron remnants on endothelial function by minor dietary lipid components

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Abstract
There is emerging evidence that minor components from dietary oils can modulate or even improve events occurring in the development of atherosclerosis. One of the earliest events of the atherosclerotic process is endothelial dysfunction, which is an activation of the endothelium manifested by an increase in pro-inflammatory molecules, such as cytokines and adhesion molecules. Chylomicron remnants, such as LDL (low-density lipoprotein), are considered to be pro-atherogenic lipoproteins because they interact with endothelial cells and macrophages, increasing endothelial dysfunction mainly by the disturbance of the redox state in the cell. However, chylomicrons are, at the same time, the natural carriers of dietary lipids in plasma, which gives minor lipid components the opportunity to interact with the cells implicated in endothelial dysfunction and atherogenesis. Some of these components are known to exhibit antioxidant, anti-inflammatory and anti-atherogenic effects in vitro, even forming part of triacylglycerol-rich lipoproteins, such as chylomicrons.

Atherosclerosis, endothelial dysfunction and diet
Endothelial dysfunction is one of the first events occurring during the development of atherosclerosis. When dysfunctional, the endothelium increases flow disturbances due to improper vasoreactivity and it initiates inflammatory responses by releasing pro-inflammatory cytokines and adhesion molecules [1]. Circulating monocytes are attracted by these molecules and adhere to the endothelium, from which they transmigrate to the subendothelial space. Once within the endothelium, monocytes differentiate into macrophages, which scavenge oxidized LDL (low-density lipoprotein) and CMRs (chylomicron remnants), becoming foam cells and contributing to the formation of the atheroma [2].

The great number of epidemiological studies developed in different countries constitute a firm and reliable base in support of the modulation of the development of atherosclerosis by dietary components. Among dietary patterns, the so-called Mediterranean diet has been revealed as one of the healthiest. The Seven Countries Study [3] concluded that a diet poor in saturated fat and rich in mono-unsaturates, in terms of olive oil, was the cause of the lower incidence of cardiovascular disease and cancer in the Mediterranean countries. Recently, the PREDIMED (Prevenci´on con Dieta Mediterr`anea) study has demonstrated in a population of 772 patients that risk factors for cardiovascular disease can be reduced by a Mediterranean-style diet rich in VOO (virgin olive oil) or nuts [4].

Chemical composition of dietary oils
Dietary oils can be structured into two fractions from a chemical point of view. The major fraction constitutes 98–99% of the oil and is mainly composed of the saponifiable compounds, such as TAGs (triacylglycerols). The term ‘saponifiable’ refers to the ability of glyceridic compounds to form soaps when treated with a base. Minor compounds account for the remaining 1–2%, comprising the unsaponifiable fraction, phenolics and waxes, which can confer important biologic activities on the dietary oil. Some of the most relevant minor components are hydrocarbons, tocopherols, fatty alcohols, phytosterols, triterpenic dialcohols and phenolic compounds.

Among hydrocarbons, squalene is present in a few oils, such as olive, peanut or shark liver oils; however, other hydrocarbons such as β-carotene and lycopene are the most important as they are precursors of vitamin A and antioxidants respectively. Sterols are present in both animal and plant dietary lipids. In animals, cholesterol is the most abundant whereas phytosterols are constituted mainly by β-sitosterol, campesterol, Δ7-stigmasterol, stigmasterol, spinasterol and avenasterol. Phytosterols have acquired great interest in the last few years because of their recognized hypocholesterolaemic effect [5]. The main tocopherol present in dietary oils is α-tocopherol but relevant amounts of

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β-, γ- and δ-tocopherols are also present. Triterpenic dialcohols and acids are incorporated into second pressing olive oils (pomace olive oil), the final concentration being higher than that in VOO.

Most dietary oils require a refining process before commercialization, which involves removal of most hydrophilic components. VOO constitutes an exception to this rule as it is obtained from the fruit and/or leaves of the olive tree as a ‘juice’ and does not need to be refined. For this reason it contains phenolic compounds, of which other dietary oils are usually devoid. Phenolic compounds have emerged as potent antioxidants present in VOO. Among these compounds, oleuropein itself and its derivative hydroxytyrosol have been reported to have a protective role against oxidative stress markers in vivo [6].

Incorporation of lipid components into chylomicrons

Minor lipid components from dietary fats are absorbed in the intestine, similarly to other lipids, and are incorporated into CMs (chylomicrons) for their transport in plasma. The process of CM assembly in enterocytes involves two steps. The size of the nascent CM particles is partially determined by the size of the lipid droplets they fuse with in the enterocyte. Likewise, the size and composition of the lipid droplets depend on the lipid composition of the diet [7]. We have reported different postprandial responses to oleic acid-rich oils, which were attributed to differences in TAG molecular species or minor components compositions [8].

Minor lipid compounds are not equally absorbed and/or incorporated into CMs, which affects their bioavailability. It has been suggested that phytosterols can be efficiently absorbed but are not incorporated into CMs because they are not efficiently esterified [9]. Phytosterols are not suitable substrates for acyl coenzyme A:cholesterol acyl transferase, the enzyme responsible for cholesterol esterification in the intestine and the liver [10]. Whereas up to 90% of cholesterol is esterified in rat enterocytes, only 20% of phytosterols are transformed into esters [11]. However, Gylling et al. [12] have reported that in humans sitosterol esterification is sterified in rat enterocytes, only 20% of phytosterols are not efficiently esterified [9]. Phytosterols are not suitable for incorporation into CMs, which affects their bioavailability.

Phytosterols are incorporated into CMs, only 25% of lycopene is secreted in the form of CMs [16]. However, the extent of cellular uptake is more similar (15–18%), indicating that lycopene differences in carotenoid structure account for their ability to be incorporated into CMs [16].

Once CMs are in the bloodstream there is a transfer of apolipoproteins from HDL, such as apo C-II, which acts as a cofactor for lipoprotein lipase, enhancing its binding with the CMs on the endothelial surface of blood vessels and unloading of its massive TAG content [17]. It has been demonstrated that CMs are not completely hydrolysed and that they still contain approx. 50% of their initial lipid load when they leave the bloodstream [18]. Once hydrolysed, CMs present the necessary size to be taken up by the liver by the action of the apoE (apolipoprotein E)-dependent receptors LDLr (LDL receptor) and LRP (LDLr-related protein) [19]. This is the way most of the dietary cholesterol and non-glycercide lipids reach the liver pool [20]. The incorporated lipids can be stored or released again into circulation in the form of VLDL.

Since CMR particles can enter into the sub-endothelial space of the artery wall, the lipids they carry have the opportunity to interact directly with cells in the tissue. In vitro experiments have shown that CMs can induce an enhancement of the expression of E-selectin and VCAM-1 (vascular cell adhesion molecule-1) in endothelial cells [21]. Additionally, recent findings have shown that CRLPs (CMR-like particles) strongly induce COX-2 (cyclo-oxygenase-2) expression in human endothelial cells, and suggest that the type of dietary fat carried in the particles influences their ability to induce COX-2 [22]. CRLPs can enhance endothelial production of the vasoconstrictor molecule TXA2 (thromboxane A2) [22], suggesting that these particles may promote an imbalance in the production of vasodilator and vasoconstrictor mediators and hence impact on the pro-atherogenic properties of the endothelium. Actually, it has been demonstrated that remnant lipoproteins inhibit endothelium-dependent relaxation of aortas [23] as well as NO (nitric oxide)-mediated endothelium-dependent vasodilation [24]. CMRs cause extensive lipid accumulation in macrophages that have invaded the sub-endothelial space [25]. Dietary lipids carried in these particles therefore have the potential to induce and modulate macrophage foam cell formation. CMRs are taken up by macrophages through multiple pathways, being one of the most important scavenger receptors [26]. Up-regulation of macrophage scavenger receptor activity has been related to decreased tumour necrosis factor-α production, which occurs after incubation with CRLPs [27].

Dietary lipids and endothelial function

Despite the widespread interest in the influence of nutrients on endothelial function, the effects of dietary oils have not been extensively addressed [28]. In vitro studies support favourable effects of fish oil on endothelial function, but results from in vivo studies are less consistent and all effects are attributed to n-3 fatty acids [29]. VOO has been
shown to improve endothelial function in diabetic [30] and hypercholesterolaemic patients [31]. We recently incubated endothelial cells with postprandial TRLs (triacylglycerol-rich lipoproteins) derived from the intake by healthy subjects of meals containing VOO and VOO enriched in its unsaponifiable fraction [EVO (enriched VOO)] to a final concentration of 2.4% [32]. We found a reduction in the production of PGE2 (prostaglandin E2) and TXB2 (thromboxane B2) after the incubation with EVO-TRL, compared with VOO, but no effect on NO production. These results suggest that minor components from VOO that are transported postprandially in TRL may have favourable effects on endothelial function by improving the balance between vasoprotective and prothrombotic factors released by endothelial cells. We consider it very unlikely that VOO phenolics were responsible for the effects observed in this study, because its concentration in VOO and EVO was very similar and because due to their hydrophilic nature they are not transported by TRL. Therefore we suggested that tocopherols, sterols or terpenoids might be responsible for the effects observed.

Vitamin E has a protective role against the attacks of free radicals but recently, non-antioxidant functions of vitamin E have been proposed. Apart from protecting LDL from lipid peroxidation [33], α-tocopherol has an inhibitory effect on LDL- and cytokine-induced production and expression of adhesion molecules [34] and adhesion of monocytes to endothelial cells [35] (Figure 1). However, this effect does not seem to be mediated by the NF-κB (nuclear factor κB) [36]. Vitamin E can also modulate eicosanoid metabolism in endothelial cells as it can restore reduced prostaglandin I2 synthesis [37] and inhibit 5-LOX (5-lipoxygenase) and COX-2 activities [38]. Meydani et al. [39] demonstrated that α-tocopherol can eliminate the increase in PGE2, TXA2 and TXB2 by reducing the activity of COX-2 in LPS (lipopolysaccharide)-stimulated macrophages from aged mice. This effect was found not to be due to regulation of COX-2 transcription or translation, but to the effect of α-tocopherol scavenging hydroperoxides and NO*, which leads to lower production of peroxynitrites. There are data suggesting that peroxynitrites may modulate COX-2 activation via Ca2+-dependent PLA2 (phospholipase A2) activity and AA (arachidonic acid) release [40].

Despite the increasing evidence of the hypocholesterolaemic effect of phytosterols [8], very little is known about their effect on vascular function. de Jongh et al. [41] administrated a mixture of phytosterols (β-sitosterol, campesterol, stigmasterol and others) to 41 children with familial hypercholesterolaemia, finding a reduction in LDL-cholesterol but no effect on endothelial dysfunction as measured by flow-mediated dilation. However, Moreno [42] incubated PMA-stimulated RAW 264.7 macrophages with β-sitosterol, and observed a reduction in ROS (reactive oxygen species) production and AA release (Figure 1). ROS modulation may regulate the release of AA by PLA2, as well as the induction of COX-2 through NF-κB activation. By this mechanism β-sitosterol might reduce PGE2 and LTB4 (leukotriene B4) production by macrophages, as observed in this study. Subsequently, this author reported that β-sitosterol can regulate the glutathione (GSH) redox cycle, enhancing GSH peroxidase and superoxide dismutase activities, hence decreasing superoxide anion levels although no ROS scavenger activity was found.

The potential therapeutic importance of triterpenoids has not been extensively studied. *In vitro* oleanolic acid inhibits 5-LOX and COX-2 activities [43,44], therefore reducing the production of PGE2 and LTB4 (Figure 1). Additionally, it has been shown to inhibit the generation of
the superoxide anion by human neutrophils, which might occur through the protein–kinase-independent pathway [45]. Although anti-inflammatory activities have been attributed to the triterpenic dialcohol erythrodial, its mechanism of action is still unknown. Erythrodial is able to reduce the oedema caused by PMA in mice, with a possible action on PLA₂ [46]. We recently developed a study to evaluate the properties as vasodilatory agents of oleanolic acid and erythrodial and to determine their mechanism of action [47]. Results from this work introduced the first in vitro evidence that oleanolic acid and erythrodial evoke an endothelium-dependent vasorelaxation in rat aorta, and suggested that the mechanism of relaxation is mediated by the endothelial production of NO. Thus it was concluded that oleanolic acid and erythrodial intake can protect against endothelial dysfunction.

Phenolic compounds are not lipid components and are not transported in plasma within CMs but are present in VOO at concentrations at which they can influence endothelial function, so they deserve a mention here. The radical VO...

Conclusions and future investigations
The increasing interest in food supplementation with nutraceuticals is boosting research into their effects on pathologies such as atherosclerosis. Some minor lipid compounds, which can be ingested as part of dietary oils, have shown beneficial effects on endothelial dysfunction, one of the first events occurring in atherogenesis. However, only few studies have addressed the influence of these compounds as part of CMs and their remnants, the vehicles in which they are transported in plasma due to their lipophilic nature. In this way, minor lipid components can modulate or reduce the detrimental effects of pro-atherogenic CMRs and potentially improve endothelial function. Since some of these minor compounds are poorly absorbed and/or incorporated into CMs there is need of bioavailability studies and randomized cross-over trials to demonstrate that they can exert their effects when they are ingested as part of dietary oils or food supplements.

References

Received 27 September 2006