

Invited commentary

Atherosclerosis and conjugated linoleic acid

Atherosclerosis can be defined as the development of abnormal fat deposits in the artery wall. Atherosclerotic lesions are believed to progress through a focal, lipid-filled foam cell stage (fatty streak) into an advanced, complicated lesion that contains abundant extracellular cholesterol ester in the atheromatous gruel within the arterial intima. Weakening of the arterial wall and/or rupture of complicated lesions may presage clinical complications. Atherosclerosis is known to occur in many arteries throughout the body, although the association between atherosclerosis extent in different arteries is variable (Wolfe *et al.* 1994; Rudel *et al.* 1995*a,b*). In human subjects, atherosclerosis in the coronary arteries is the underlying cause of CHD with its associated mortality from myocardial infarction. The literature contains many references to effects of specific fatty acids on the development of atherosclerosis or CHD. The strongest case can be made when clinical and epidemiological data in human subjects is supported by data in appropriate animal models. Study in relevant animal models facilitates assignment of molecular mechanisms.

A classic example is that of linoleic acid. Diets enriched in this fatty acid have long been shown to be beneficial in reducing CHD risk in human subjects (Morris *et al.* 1977; Shekelle *et al.* 1981), and have been shown to protect against the development of coronary artery atherosclerosis in monkeys (Rudel *et al.* 1995*a,b*) and aortic atherosclerosis in transgenic mice (Rudel *et al.* 1998). Efforts to reduce LDL-cholesterol concentration and modify LDL particle composition both appear related to the beneficial effects. Work done in hamsters (Spady & Dietschy, 1988; Spady *et al.* 1993) has suggested a potential mechanism for the beneficial effect of dietary linoleic acid in lowering LDL-cholesterol concentrations. Diets containing enrichments of this polyunsaturated fatty acid (PUFA) appear to limit down-regulation of hepatic LDL receptors by dietary cholesterol, perhaps by limiting the availability of hepatic cholesterol to a putative regulatory pool. Resulting plasma LDL clearance rates may be higher and LDL-cholesterol concentrations lower. LDL composition may also be important in the linoleic acid effect (Rudel *et al.* 1997). In his original prediction that essential PUFA would protect against atherosclerosis, Sinclair (1956) suggested that more-saturated cholesterol esters may be less readily disposed of and be more atherogenic. Subsequent clinical studies have shown inverse associations between the proportion of plasma cholesterol ester as cholesteryl linoleate and adverse CHD outcome (Lawrie *et al.* 1961; Kingsbury *et al.* 1969; Kirkeby *et al.* 1972).

While this information about positive effects on atherosclerosis of diets containing linoleic acid is available, a potential down-side also exists. A popular theory of atherosclerosis postulates that increased oxidation of low density lipoproteins (LDL) predisposes to increased CHD (Steinberg *et al.* 1989). The enrichment of LDL with lipids rich in linoleic acid demonstrably predisposes LDL particles to oxidative modification *in vitro* (Reaven *et al.* 1993; Thomas *et al.* 1994), and yet less atherosclerosis has been demonstrated in this setting as mentioned above. While this apparent paradox does not invalidate the hypothesis that LDL oxidation is important in atherosclerosis, it does suggest that the phenomenon of LDL oxidation, by itself, is not sufficient to explain atherogenesis. Linoleic acid apparently has benefits to atherosclerosis that override any increase in susceptibility to oxidation.

An isomer of linoleic acid that also may have important consequences for atherosclerosis, as well as cancer, is conjugated linoleic acid (CLA). Actually, CLA represents several positional isomers of linoleic acid, including what may be the most common isomer, $\Delta 9$ -*cis*, $\Delta 11$ -*trans* octadecadienoic acid (Banni & Martin, 1998). While CLA is not a major constituent of any fat source as linoleic acid is, it appears to be present in small amounts (typically < 2 %) in many fat sources. This fatty acid appears to be a product of a biohydrogenation reaction catalysed by an enzyme, linoleate isomerase (*EC* 5.2.1.5), present in the bacteria of ruminant animals (Kepler *et al.* 1966, 1970). While some CLA may also be formed during chemical hydrogenation reactions, it appears that it is present in higher proportions in dairy products than in vegetable fats (Chin *et al.* 1992). Availability of CLA in milk fat may be a function of the diet, with higher proportions being present when the animals' diets contain more PUFA. The CLA present in human tissue and blood plasma is apparently derived from the diet, although this is not yet established with certainty. Rats have an enzyme in liver that can convert the $\Delta 11$ -*trans* octadecenoic acid, itself one of the common positional isomers of *trans* fatty acids, to the $\Delta 9$ -*cis*, $\Delta 11$ -*trans* octadecadienoic acid (Banni & Martin, 1998). It is unknown if this pathway is present in human liver, but if it were present, at least some of the body's CLA could be formed from some of the *trans* fatty acids in partially hydrogenated fats, for example. In any case, it is important to understand if CLA is indeed beneficial and not harmful.

Numerous studies have documented an anticarcinogenic activity for CLA in animal models (reviewed by Banni & Martin, 1998). However, a potential role for CLA in

atherogenesis has only been examined in three studies, including the paper by Munday, Thompson and James in this issue (Munday *et al.* 1999). In the first two studies, rabbits were fed on a modestly atherogenic diet enriched with 5 g CLA/kg for 22 weeks (Lee *et al.* 1994) while three levels of CLA (0.06, 0.11 and 1.1% of energy) were tested in hamsters fed on atherogenic diets for 8 weeks (Nicolosi *et al.* 1997). Both of these studies appeared to show that CLA protected against the development of early arterial lipid accumulation, although the benefits when CLA was present in the diet were small and no dose–response effect was apparent in the hamster study. In both studies, only very early aortic atherosclerosis was studied, although the study in rabbits represented a more substantial population of atherosclerotic lesions than the study in hamsters. The conclusion from the Munday, Thompson and James study in the C57Bl/6 mouse, fed on atherogenic diets with either 2.5 or 5.0 g CLA/kg for 16 weeks, was that relatively more aortic leaflet lipid deposition occurred, again without a dose–response effect. As in the other studies, differences among experimental groups in arterial lipid deposition were small, with the effect of the diet containing the higher level of CLA not being significantly different from the effect of the control diet.

In other words, the studies that have been completed on the effects of CLA on atherosclerosis are few and have not been definitive. All three studies have focused only on early atherosclerotic lesion development. Three different animal models have been examined, and species differences may contribute to the uncertainty. At this point it is impossible to predict if CLA will have any effect on atherosclerosis in humans.

It will be important for future studies to demonstrate CLA-mediated differences in more well developed atherosclerotic lesions, and to identify effects on coronary artery atherosclerosis so that it becomes possible to know whether or not CLA could prevent (or worsen) complications of more clinically relevant atherosclerosis. It will be important to get data in human populations on the effects of CLA on CHD. However, the effects of CLA, *per se*, in human subjects may be difficult to identify due to the confounding factors (including saturated fat and cholesterol) in the food sources most enriched in CLA. Therefore, more work in animal models to demonstrate a consistent effect of CLA on atherosclerosis will be needed in the interim. In each of the studies completed to date, dietary CLA appears to have decreased total plasma cholesterol concentrations without decreasing HDL-cholesterol concentrations, setting up a potentially more atherogenic lipid profile. This suggests that CLA could alter the development of atherosclerosis via effects on hepatic cholesterol metabolism. Use of animal models to examine potential mechanisms for CLA to alter liver cholesterol metabolism and thus plasma cholesterol, such as those discussed above for linoleic acid, are therefore indicated. Finally, of course, it will be necessary to learn if all of the various positional isomers of CLA will have equal effects on any of these endpoints.

In short, the potential role(s) of CLA in the pathobiology of atherosclerosis remains to be determined as our present knowledge is quite limited. The final statement made by

Munday, Thompson and James is correct. CLA currently cannot be regarded as anti-atherogenic. Nor can they be regarded as atherogenic.

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