Invited commentary

Good COP, bad COP: an unsolved murder. Are dietary cholesterol oxidation products guilty of atherogenicity?

The link between elevated plasma cholesterol concentrations and atherosclerosis is well established, but the role of cholesterol oxidation products (COP), also termed oxysterols, is still controversial. Relevant, extensive reviews include those of Brown & Jessup (1999), Schroepfer (2000), Björkhem & Diczfalusy (2002) and Garcia-Cruset et al. (2002). Oxysterols appear to be atherogenic in some, but not all, studies in animal models; in vitro, oxysterols exhibit various effects, many of which are potentially pro-atherogenic, including toxicity to macrophages, smooth muscle cells and endothelial cells (for reviews, see Brown & Jessup, 1999; Schroepfer, 2000; Björkhem & Diczfalusy, 2002; Garcia-Cruset et al. 2002). Death of macrophage foam cells leads to the development of the lipid core of advanced atherosclerotic lesions whilst death of smooth muscle cells thins the fibrous cap, and these changes destabilise lesions, predisposing them to rupture with thrombogenic consequences. Impairment of endothelial cells leads to loss of barrier function and promotes cell adhesion and coagulation. The results of a new study, published in the present issue of the British Journal of Nutrition (Ando et al. 2002), in an animal model provides evidence that dietary oxysterols are non-atherogenic.

Oxysterols usually occur at low levels accompanied by high concentrations of the parent cholesterol. Oxysterols have long been known to occur in samples of cholesterol that have been exposed to oxygen from the air as a result of prolonged storage or by heating. There are many reports of oxysterols in human atherosclerotic lesions (Brown & Jessup, 1999). Six oxysterols identified in human advanced lesions totalled 18 μg/mg cholesterol (sum of average levels of each individual oxysterol), whereas in macroscopically normal specimens of artery distant from lesions, oxysterols were about 10-fold less abundant (Garcia-Cruset et al. 2001). In advanced lesions, 27-hydroxycholesterol is the most abundant oxysterol (Garcia-Cruset et al. 2001). In advanced lesions, 27-hydroxycholesterol is the most abundant oxysterol (Garcia-Cruset et al. 2001). 27-Hydroxycholesterol levels in lesions increase with lesion severity (Carpenter et al. 1995; Garcia-Cruset et al. 2001). In advanced lesions, 27-hydroxycholesterol is the most abundant oxysterol (Garcia-Cruset et al. 2001) and it is more abundant in the lipid core than in the fibrous cap (Garcia-Cruset et al. 1999). 27-Hydroxycholesterol is also the most abundant oxysterol in human plasma (Babiker & Diczfalusy, 1998).

27-Hydroxylase is believed to constitute a cholesterol removal mechanism from extra-hepatic cells. 27-Hydroxycholesterol leaves cells more readily than the parent cholesterol and, moreover, 27-hydroxycholesterol can oxidise 27-hydroxycholesterol further to form 3β-hydroxycholestenoic acid, which exits even more readily (Babiker et al. 1999). There is a flux of 27-hydroxycholesterol and 3β-hydroxycholestenoic acid from the tissues to the liver, where these compounds are converted to bile acids (Björkhem & Diczfalusy, 2002). 27-Hydroxylase can also act on 7-oxysterols as substrates, preventing macrophages accumulating 7-ketocholesterol (Brown et al. 2000), which might constitute an oxysterol-removal mechanism (analogous to that for cholesterol) from atherosclerotic lesions. Plasma oxysterols are associated with lipoproteins,
apart from \(3\beta\)-hydroxycholestenolic acid, which is in
the lipoprotein-free fraction (Björkhem & Diczfalusy, 2002).

Many oxysterols can be produced non-enzymatically, as
illustrated by Ando et al. (2002), including \(\Delta 7\) and
\(\Delta 7\beta\)-hydroxycholestanols, \(\Delta 5\alpha,6\alpha\)- and \(\Delta 5\beta,6\beta\)-epoxychole-
sterols, cholestanetriol, 7-ketocholesterol (also termed
7-oxocholesterol) and 25-hydroxycholesterol. \(\Delta 7\alpha\)-Hydroxy-
cholesterol can also be produced enzymatically by hepatic
7\(\alpha\)-hydroxylase. Plasma \(\Delta 7\alpha\)-hydroxycholesterol is at least
derived from ‘leakage’ from the liver (Björkhem & Diczfalusy, 2002). 27-Hydroxycholesterol is a purely
enzymatic product (see earlier). Recently, a sterol
25-hydroxylase that produces 25-hydroxycholesterol has
been characterised (Lund et al. 1998). \(\Delta 7\alpha\)- and \(\Delta 7\beta\)-
Hydroxycholestanols and 7-ketocholesterol can arise from
decomposition of \(\Delta 7\alpha\)- and \(\Delta 7\beta\)-hydroperoxycholestanols,
which are produced by free radical oxidation of choles-
terol. \(\Delta 7\beta\)-Hydroxycholesterol is usually more abundant
than its \(\Delta 7\alpha\)-isomer because the former is less sterically
hindered. Cholestanetriol is believed to arise from opening
of the epoxide ring of 5,6-epoxycholestanols.

The 7-position of cholesterol is vulnerable to free radical
attack because abstraction of a \(\text{H}\) atom from this position
produces an allylic radical, i.e. the unpaired electron is
resonance-stabilised by the C–C double bond of the
cholesterol molecule. Oxidation of cholesterol is markedly
promoted by the presence of polyunsaturated fatty acids,
where peroxidation of the latter (either non-enzymatically
or by the action of lipoxygenase) gives rise to polyunsatur-
ated fatty acid-derived radicals that proceed to attack
cholesterol at the 7-position. This occurs in oxidation of
LDL in vitro by macrophages or by Cu\(^{2+}\) ions (Carpenter
et al. 1994). Oxidised LDL has been detected in athero-
sclerotic lesions both in human subjects and apolipoprotein

There have been many attempts to model human athero-
sclerosis using animals. Imai et al. (1976) produced
evidence that the atherogenicity and angiototoxicity of diet-
ary cholesterol to rabbits was due to oxysterols present
as auto-oxidation impurities in the cholesterol used to sup-
plement the diets. Numerous studies have investigated the
atherogenicity of dietary oxysterols in animals (see reviews
mentioned earlier), but the overall effect of oxysterols was
unclear, some studies suggesting an atherogenic effect,
whilst others showed no effect or even an anti-atherogenic
effect.

Recently, dietary oxysterols were shown to be athero-
genic by Staprans et al. (2000) in a strain of mice
genetically engineered to be deficient in ApoE, an animal
model in which plasma cholesterol levels are elevated
due to the accumulation of VLDL and chylomicon rem-
nants. In mice consuming the oxysterol-supplemented
diet, oxysterol levels increased in plasma, but no measure-
ments were made of oxysterols in the artery (aorta) wall.

The new study in ApoE-deficient mice (Ando et al.
2002) assessed the lesion volume (as lesion area in trans-
verse sections) in the aortic root, as well as levels of
cholesterol and oxysterols in serum, liver and aorta, result-
ing from diets supplemented with cholesterol (200 mg/kg
diet), or with oxysterols (200 mg/kg diet) produced by
heating cholesterol, or without supplements (control).
Neither the cholesterol-supplemented diet nor the
oxysterol-supplemented diet resulted in significant change
in size of lesions or aortic cholesterol concentration com-
pared with the control diet. Except for 27-hydroxychole-
terol, levels of oxysterols increased significantly in the
serum and livers of mice supplemented with dietary oxy-
sterols, though the percentage increase in liver oxysterol
levels was less dramatic than in serum. In the aortas,
by contrast, only 7-ketocholesterol and cholestanetriol
increased significantly, but not as markedly as in serum.
Setting aside 27-hydroxycholesterol (an entirely enzymatic
COP, absent from the dietary supplement of oxysterols),
the ranking order of relative abundances of the individual
oxysterols was different in diet, serum, liver and aorta.
Oxysterols are thus not simply taken up en masse from
diet to serum to tissues, and the profile of the dietary oxy-
sterols is modulated in vivo, almost certainly involving
differential uptake, synthesis, metabolism and removal.
Oxysterols formed in vivo are \(\Delta 7\alpha\) and \(\Delta 7\beta\)-hydroxychole-
sterols, 7-ketocholesterol, and 24-, 25- and 27-hydroxycho-
lesterols, demonstrated in rats by \(^{18}\text{O}\) 2 inhalation (Breuer &
Björkhem, 1995).

Surprisingly, among the results of Ando et al. (2002) is
the apparent lack of aortic uptake of oxysterols, apart from
7-ketocholesterol and cholestanetriol, which might both be
formed at least partly in situ in the artery wall. It is often
tacitly assumed in biological studies that plasma (or serum)
and tissue levels of a substance will reflect each other
to some extent, and the study of Ando et al. (2002)
strikingly illustrates that this is not always true. Another
such example is in the human disease cerebrotendinous
xanthomatosis, in which plasma cholesterol levels are
usually normal, but tissue levels of cholesterol are
abnormally high due to a genetic deficiency of sterol 27-
hydroxylase (a cholesterol removal mechanism; see
earlier); cerebrotendinous xanthomatosis sufferers often
develop premature atherosclerosis (Björkhem & Diczfalusy,
2002).

The reason for the apparent discrepancy between the
findings of Ando et al. (2002) and Staprans et al. (2000),
i.e. dietary oxysterols were non-atherogenic in the former
but atherogenic in the latter, is unknown. Whilst there
were some differences in conditions between the two
studies, the levels of oxysterols in the diets were approxi-
mately the same, and so were the serum cholesterol con-
centrations. The study by Staprans et al. (2000) involved
a 4-month dietary supplementation period whereas that of
Ando et al. (2002) was 8 weeks. Another possible import-
ant difference is as follows. Ando et al. (2002) oxidised
cholesterol then used column chromatography (silica gel)
to remove most of the residual cholesterol and isolate an
oxysterol fraction that was used to supplement the diet.
Staprans et al. (2000), in contrast, did not fractionate
the oxidised cholesterol, so that the oxysterols in the diet
were accompanied by unoxidised parent cholesterol. Diet-
ary cholesterol might act as a ‘vehicle’ for dietary oxy-
sterols, as serum levels of oxysterols, recalculated as \(\mu\text{g/mg}\)
cholesterol for the study of Ando et al. (2002), appeared
somewhat lower than those of Staprans et al. (2000). More-
ever, some uncharacterised pro-atherogenic species might
have been lost in the fractionation process of Ando et al. (2002).

ApoE-deficient mice have become widely used over the past decade to model atherosclerosis (Breslow, 1996; Moghadasian et al. 2001). ApoE normally mediates uptake of remnant lipoproteins by several receptor systems in the liver. If ApoE is deficient or defective, VLDL and chyomicron remnants accumulate in the plasma due to poor clearance, and the plasma cholesterol concentration is markedly elevated. VLDL, chyomicrons and their remnants are triacylglycerol-rich lipoproteins, normally containing ApoE, and they also contain cholesterol, cholesteryl ester and phospholipid. The VLDL that accumulates if ApoE is deficient or defective is termed bteryl ester and phospholipid. The VLDL that accumulates containing ApoE, and they also contain cholesterol, cholesterol remnants are triacylglycerol-rich lipoproteins, normally poor clearance, and the plasma cholesterol concentration is markedly elevated. VLDL, chylomicrons and their remnants are triacylglycerol-rich lipoproteins, normally containing ApoE, and they also contain cholesterol, cholesteryl ester and phospholipid. The VLDL that accumulates if ApoE is deficient or defective is termed β-VLDL, containing higher levels of cholesteryl ester than normal VLDL. In man, this condition is termed type III hyperlidaemia and results in premature atherosclerosis. The lesions of ApoE-deficient mice range from early (fatty streak) to advanced (fibrous plaque), and dietary supplementation with fat, cholesterol and cholic acid accelerates lesion progression. Lipid-rich, unstable advanced lesions with thinned fibrous caps were reported in ApoE-deficient mice aged 17 months (Moghadasian et al. 2001), i.e. older than the mice in the studies of Ando et al. (2002) and Staprans et al. (2000), where the lesions were at an earlier stage, described as fatty streaks by Staprans et al. (2000). ApoE is produced by the liver and it is also secreted by macrophages. Bone-marrow transplantation from normal mice into ApoE-deficient mice diminished the progression of atherosclerosis and reduced serum cholesterol levels (Van Eck et al. 2000).

Oxidative stress appears to be involved in atherosclerosis in ApoE-deficient mice and in human subjects (Hayek et al. 1994; Pratico, 2001). Recently, Rosenblat & Aviram (2002) produced evidence for oxysterol-induced activation of NAPDH-oxidase in ApoE-deficient mouse macrophages, enhancing LDL oxidation in vitro. Dietary supplementation with natural antioxidants (vitamin E or vitamin E plus ubiquinone) was anti-atherogenic in ApoE-deficient mice (Pratico et al. 1998; Thomas et al. 2001). In the latter study, the antioxidant supplementation lowered aortic levels of lipid hydroperoxides and 7-ketocholesterol when expressed per mg protein, but when standardised for cholesterol, the lowering of 7-ketocholesterol was not statistically significant. Ando et al. (2002) noted that all the diets used in their study contained the artificial antioxidant tert-butylhydroquinone, which might have counteracted the potential pro-oxidant, pro-atherogenic effects of the oxysterols.

Oxysterols can act as regulators of cholesterol homeostasis in various ways (Brown & Jessup, 1999; Björkhem & Diczfalusy, 2002), which might counteract the potential atherogenic effects of the oxysterols. The down-regulation by oxysterols of hydroxymethyl glutaryl CoA reductase (a key enzyme in cholesterol biosynthesis) is among these. In addition, some oxysterols are activating ligands for liver X receptors, transcription factors regulating several genes important in cholesterol homeostasis, e.g. the ABCA1 cholesterol transporter pathway, resulting in efflux of cholesterol from macrophages. Oxysterols themselves might be similarly exported along with cholesterol, constituting a self-limiting mechanism for oxysterol levels in lesions. Furthermore, the toxicity of several of the oxysterols to macrophages in vitro was inhibited by cholesterol (Clare et al. 1995). Such an effect, if true in vivo, where cholesterol is abundant, might at least partly explain the lack of atherogenicity of dietary oxysterols in the study of Ando et al. (2002). Serum oxysterol levels in human subjects are considerably lower (whether expressed per litre serum or per mg cholesterol) than those achieved in ApoE-deficient mice receiving dietary oxysterol supplementation (Staprans et al. 2000; Ando et al. 2002).

The results of Ando et al. (2002) will be reassuring to some, as providing evidence for the defence of dietary oxysterols, alias COP, on this occasion acquitting them of promoting atherosclerosis in ApoE-deficient mice under the conditions of their study. As to whether dietary oxysterols are atherogenic in man, the jury is still out.

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References


