Antioxidant Vitamins and Lipid Therapy
End of a Long Romance?
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Abstract—During the past decade, the perception flourished that lipid and antioxidant therapy were 2 independent avenues for cardiovascular protection. However, studies have shown that commonly used antioxidant vitamin regimens do not prevent cardiovascular events. We found that the addition of antioxidant vitamins to simvastatin-niacin therapy substantially blunts the expected rise in the protective high density lipoprotein (HDL)2 cholesterol and lipoprotein(A-I) subfractions of HDL, with apparent adverse effects on the progression of coronary artery disease. To better understand this effect, 12 apolipoproteins, receptors, or enzymes that contribute to reverse cholesterol transport have been examined in terms of their relationship to HDL2 and lipoprotein(A-I) levels and the potential for antioxidant modulation of their gene expression. Three plausible candidate mechanisms are identified: (1) antioxidant stimulation of cholesteryl ester transfer protein expression/activity, (2) antioxidant suppression of macrophage ATP binding cassette transmembrane transporter A1 expression, and/or (3) antioxidant suppression of hepatic or intestinal apolipoprotein A-I synthesis or increase in apolipoprotein A-I catabolism. In summary, antioxidant vitamins E and C and /H9252 -carotene, alone or in combination, do not protect against cardiovascular disease. Their use for this purpose may create a diversion away from proven therapies. Because these vitamins blunt the protective HDL2 cholesterol response to HDL cholesterol–targeted therapy, they are potentially harmful in this setting. We conclude that they should rarely, if ever, be recommended for cardiovascular protection. (Arterioscler Thromb Vasc Biol. 2002;22:1535-1546.)

Key Words: antioxidant vitamins ■ lipid therapy ■ atherosclerosis

The Romance
Over the past 15 years epidemiological,1,2 basic biological,3–5 and experimental atherosclerosis6–9 studies have supported the idea that antioxidants can protect against atherosclerosis by limiting LDL oxidation and, thus, the macrophage scavenger receptor (SR)-mediated accumulation of cholesteryl ester (CE) in the lipid-rich necrotic center of the plaque. Increased foam cell density9 and inflammatory activity10,11 and large core lipid size9 (but not stenosis severity12,13) have been identified as high-risk plaque characteristics. Although other mechanisms for plaque lipid accumulation have been described,14 oxidized LDL has provided an attractive hypothetical therapeutic target.15

In the same time period, clinical trials have found substantial risk reduction by pharmacological lowering of LDL cholesterol (LDL-C).16,17 The perception flourished that lipid lowering and antioxidant therapy were 2 independent avenues for cardiovascular protection. Many medical practitioners and the public accepted, without full proof, the promise of this “natural” combination of preventive strategies. Other leaders in this area have adopted the more skeptical posture, ie, waiting for the prospective clinical trials.18,19

The Dream Fades
The antioxidant hypothesis spawned a number of large clinical trials whose results were reported between 1994 and 2000.20–24 The conclusions of these studies, briefly summarized (Table 1), are as follows: (1) There is little or no cardiovascular benefit from vitamin E in the dose ranges studied. (2) There is no cardiovascular benefit from /H9252 -carotene. (3) There is no cancer benefit from vitamin E, and at least in smokers over the short term, new cancer risk may increase with /H9252 -carotene.

Given this battering of a beautiful hypothesis with compelling negative data, a variety of explanations have been offered.25 Despite the data, physicians and the public have continued widespread antioxidant use. Part of the reasoning is that “at least it won’t hurt me.”

And Now, Incompatibility
Attracted to the hypothetical promise of the marriage of lipid therapy and antioxidant vitamins, we randomized 160 patients with coronary artery disease (CAD) and low HDL into a factorial design angiographic trial to treatment with simvastatin plus niacin or an antioxidant cocktail (vitamins E and C, β-carotene, and selenium) or to treatment with the full
combination or their placebos. The results are as follows: (1) Niacin and simvastatin lowered LDL-C, on average, by 42%, raised HDL2 cholesterol (HDL2-C) by 65% and Lp(A-I) by 76% (see Table 2). (2) When antioxidants were added to lipid therapy, HDL2-C rose by only 28%, and Lp(A-I) rose by 29% (Table 2); antioxidants alone lowered HDL2-C by 15% (P < 0.05). (3) Simvastatin plus niacin, alone, promoted regression of coronary disease, a marker of plaque lipid depletion, but when antioxidants were added to lipid therapy, the disease instead progressed, although the progression was significantly slower than that with all-placebo treatment. There was a significant adverse interaction between lipid and antioxidant therapy for this primary study endpoint. (4) Antioxidants alone resulted in a modest nonsignificant slowing of stenosis progression relative to placebo. (5) The frequency of a first major cardiovascular event was 24% for the all-placebo group, 3% for the group receiving only simvastatin plus niacin, 21% for the group receiving only antioxidants, and 14% for the group receiving the full combination.

We proposed that the diminished clinical benefits in the full combination were due to the adverse effect of antioxidants on the HDL2-C and Lp(A-I) response to lipid therapy. Thus, these results, if confirmed, suggest that a combination of antioxidant vitamins can be harmful in a patient taking lipid therapy targeted at raising HDL2-C.

With Hindsight, It Was Predictable
Probucol is a much more potent antioxidant than our vitamin cocktail. In the Probucol Quantitative Regression Swedish Trial (PQRST), all subjects had femoral atherosclerosis, received cholestyramine, and were randomized to probucol or its placebo. In the comparison of these 2 treatment groups, probucol lowered the relative level of the large buoyant HDL2b (those particles in the 9.7- to 12.9-nm size range) by 53% and the protein concentration (mainly apoA-I) of HDL2b by 67%. The increase in mean femoral artery obstruction over 3 years was significantly correlated with reduction in HDL2b protein (r = 0.44, P < 0.001) and the relative HDL2b-C value (r = 0.51, P < 0.001). In-treatment reduction of HDL2b-C had a highly significant correlation with plasma probucol concentration. In conclusion, there was a causal and biologically meaningful link between decrease in HDL2b and femoral atherosclerosis progression. Thus, a potent nonvitamin antioxidant caused a striking HDL2-C reduction and resulting atherosclerosis progression.

An early report described reduction of HDL-C (−5%, P < 0.05) and HDL2-C (−30%, P < 0.01) but not HDL3 during diet-controlled treatment of acne vulgaris with isotretinoin (13-cis-RA, where RA indicates retinoic acid). Atorvastatin, the metabolites of which are proven antioxidants at high concentration in vitro, causes a dose-dependent blunting of the HDL-C and apoAI response relative to that seen with other statins. Although it is


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HDL2-C response to lipid therapy.

Selenium, by comparison, significantly enhanced the cocktail in blunting the HDL2-C response to lipid therapy. (3)

Forty-four such patients were given 10 mg simvastatin plus 2.5 g niacin daily for 3 months and were simultaneously randomly assigned to 1 of 6 antioxidant options: (1) 800 IU vitamin E plus 1000 mg vitamin C daily, (2) 25 mg β-carotene daily, (3) 50 000 IU vitamin A daily, (4) 100 µg selenium daily, (5) the original vitamin cocktail, and (6) the original placebo. Samples were obtained at baseline and after 3 months on these combinations. The results, briefly, are as follows35: (1) The original observations on HDL-C and HDL2-C were reproduced in the substudy. (2) Three individual vitamin therapies (vitamin A, vitamins E plus C, and β-carotene) were, on their own, as effective as the full cocktail in blunting the HDL2-C response to lipid therapy. (3) Selenium, by comparison, significantly enhanced the HDL2-C response to lipid therapy.

In that small study,35 which requires confirmation, each of the vitamins comparably blunted the HDL2-C response to lipid therapy, implying a general antioxidant effect rather than a specific culprit.

Who Was to Blame?

To further examine the HDL-2 blunting effects of our antioxidant cocktail (see Table 2), we conducted a vitamin substudy for a 5-month period in patients completing the original HDL Atherosclerosis Treatment Study (HATS).28

Lipid therapy effects on HDL2-C and Lp(A-I), are significantly blunted by antioxidant vitamins.

TABLE 2. Effect of Simvastatin Plus Niacin, With or Without Antioxidant Vitamins, on Various Lipoprotein Particle Concentrations

<table>
<thead>
<tr>
<th>Plasma Variable</th>
<th>Niacin Plus Simvastatin Plus Antioxidant Placebos (n=33)</th>
<th>Niacin Plus Simvastatin Plus Antioxidant Vitamins (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C, mg/dL</td>
<td>Baseline Mean* (%) Change</td>
<td>Baseline Mean* (%) Change</td>
</tr>
<tr>
<td>HDL2-C, mg/dL</td>
<td>31.8</td>
<td>9.7 (30)</td>
</tr>
<tr>
<td>HDL3-C, mg/dL</td>
<td>3.8</td>
<td>2.3 (65)</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>28.0</td>
<td>7.4 (27)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>132</td>
<td>−57 (−42)</td>
</tr>
<tr>
<td>Lp(A-I), mg/dL</td>
<td>48.4</td>
<td>37.4 (76)</td>
</tr>
<tr>
<td>Lp(A-I, A-II), mg/dL</td>
<td>20.9</td>
<td>16.4 (81)</td>
</tr>
<tr>
<td>Apo A-I in large (9.2–11.2 nm)</td>
<td>4.6</td>
<td>8.1 (172)</td>
</tr>
<tr>
<td>Lp(A-I, A-II), mg/dL</td>
<td>225.8</td>
<td>10.7 (6)</td>
</tr>
<tr>
<td>Apo A-I in Lp(A-I, A-II), mg/dL</td>
<td>87.1</td>
<td>1.5 (3)</td>
</tr>
<tr>
<td>Apo A-I in Lp(A-I, A-II), mg/dL</td>
<td>11.9</td>
<td>4.6 (44)</td>
</tr>
</tbody>
</table>

*Mann-Whitney comparison of mean change in levels of variables between two treatment groups.

‡For the 33 patients on N+S+P, and 40 patients on N+S+A0x, who had samples at baseline, and at 12 and 24 months, and completed the study.

*Mean per-patient change from baseline to in-treatment average level (at 1 and 2 years, mg/dL, % of baseline level).

†For the 16 patients on N+A0x with HDL particle analysis at baseline and 12 months.

Lipid therapy effects on HDL2-C and Lp(A-I), are significantly blunted by antioxidant vitamins.

Our Relationship Was Just Too Complicated

A growing understanding of metabolic pathways determining HDL-C and HDL2-C levels and reverse cholesterol transport36 may help generate testable hypotheses to explain these observations. Points of potential antioxidant influence (Figure 1) include the modulation of genes expressing the ATP binding cassette (ABC) transmembrane transporter A1 (ABCA1), apoA-I, lecithin-cholesterol acyltransferase (LCAT), hepatic lipase (HL), lipoprotein lipase (LPL), endothelial lipase (EL), CE transfer protein (CETP), phospholipid (PL) transfer protein (PLTP), SR class B type I (SR-BI), and 7α-hydroxylase. Our focus in the present review is drawn to the metabolic steps that regulate the HDL2-C or Lp(A-I) particle levels.

Lipid-poor HDL of liver37 or intestinal38 origin acquires unesterified cholesterol and PL from sources including the ABCA1 transporter of the intimal macrophage (Figure 1). Only a small fraction of the cholesterol transported to the liver is derived from this cell type, but the ABCA1 pathway is its principal route of cholesterol efflux. These HDL particles mature under the influence of LCAT, PLTP, CETP, HL, and LPL into a steady-state collection of HDL particles of varying size, lipid, and apolipoprotein composition (Figure 2). Mature HDL particles dock on the hepatic SR-BI receptors, which selectively remove CE for intracellular cholesterol trafficking and bile production, and return lipid-poor apoA-I particles to the plasma for recycling or renal catabolism. In the following paragraphs, we examine the actions and regulation of these gene products.
ABCA1 Transporter

ABCA1, the mutations of which cause Tangier disease, is a 254-kDa transmembrane protein mediating cellular efflux of unesterified cholesterol, PL, and α-tocopherol via HDL-linked pathways. Transmembrane receptors/transporters are as follows: LRP, LDLR (B-E receptor), CD-36, SR-A (macrophage SRs mediating uptake of modified/oxidized LDL), ABCA1, megalin/cubulin (2 cell surface receptors working to capture and internalize lipid-poor apoA-I particles), and SR-BI (hepatic SR, a docking receptor that binds mature HDL particles, extracts CE, and releases lipid-poor apoA-I into plasma). This conceptualization reflects the work of many investigators over many years, with special thanks to 4 who have taught us a great deal and with apologies for any misrepresentations.

Apolipoprotein A-I

Overall, apoA-I, a 28.3-kDa amphipathic protein, accounts for ≈70% of the protein in HDL. ApoA-I, apoA-II, and apoA-IV are synthesized in the liver and intestine (see Lipoproteins in Health and Disease, chapter 5). Lp(A-I)s, the HDL particles containing apoA-I but not apoA-II, begin as small nascent HDL disks and lipid-poor spheres and acquire cholesterol, PL, and apolipoproteins from other lipoproteins and from cells (Figure 1). They mature (Figure 2) to become the large, more buoyant HDL2b subpopulation associated with reduced atherosclerosis risk. Lp(A-I)s carry approximately one third of the HDL-C in healthy normal individuals and less than half of that in many patients with coronary disease.

Intravenous, drug-induced, or transgenic increases in apoA-I plasma levels are correlated with increased HDL-
C 28,58 – 60 (in HATS, 28 r /H11005 0.63, P /H11005 0.0001) and with decreased atherosclerosis. ApoA-I has a structural domain that conveys protection against murine atherosclerosis that is independent of its levels.61 A deficiency of apoA-I and apoC-III synthesis results in the virtual absence of HDL-C and accelerated atherosclerosis. 62,63 Conversely, rapid apoA-I and apoA-II turnover results in low HDL-C but may protect against atherosclerosis,64 highlighting the controversy over whether HDL levels or turnover are most protective. In vivo, thyroid hormone may reduce,65 and estrogen may increase, 66 – 68 apoA-I synthesis and plasma levels. Niacin is thought to raise apoA-I levels by specifically slowing the catabolism of Lp(A-I).69

The promoter region of the apoA-I gene contains elements that respond to nuclear receptors whose activation regulates transcription of the apoA-I message.70,71 These include the antioxidant response element (ARE) and peroxisomal proliferator response element.72,73 ARE is capable of upregulating antioxidant defense genes when it is bound by a nuclear receptor activated by increased oxidative stress.72 A free radical–induced novel nuclear receptor(s) has been shown to bind to ARE, inducing a 2-fold increase in apoA-I gene transcription.72 Nuclear receptors include apoA-I regulatory protein (ARP)-1, RXR, retinoic acid receptor (RAR), and peroxisome proliferator–activated receptor (PPAR)α. ARP-1,74,75 a member of the thyroid-steroid–responsive nuclear receptor superfamily, is a nuclear receptor that suppresses apoA-I gene expression. The RXR nuclear receptors, activated by 9-cis-RA (and less so by all-trans-RA)76 stimulate apoA-I expression. PPARα activators such as fibric acids increase HDL by increasing expression of apoA-I and apoA-II genes.77,78

Apolipoprotein A-II
Little is known regarding the functions of apoA-II, a 17.4-kDa major HDL structural protein. A polymorphism in the promoter region of the apoA-II gene influences apoA-II production, visceral fat accumulation, and postprandial metabolism of large VLDLs.79 Unlike Lp(A-I), Lp(A-I, A-II) appears to promote atherosclerosis in susceptible mice,80 although this is controversial.81 In general, plasma apoA-II is thought to be proatherogenic.82 Because apoA-II and Lp(A-I, A-II) levels do not appear to be affected by simvastatin-niacin or by antioxidants (Table 2), these are not discussed further in the present review.

Lecithin-Cholesterol Acyltransferase
LCAT (see Lipoproteins in Health and Disease,24 chapter 14) is a 63-kDa glycoprotein enzyme expressed and secreted by the liver into plasma, where it is transported principally
bound to HDL particles. LCAT esterifies nearly three fourths of all plasma cholesterol and also catalyzes acylation of lysophosphatidylcholine. LCAT is activated principally by apoA-I but to a lesser extent by other HDL-bound apolipoproteins (apoC-I, apoD, apoA-IV, and apoE). Predictably, increased LCAT activity would increase HDL CE content, particle size, and HDL2-C levels. LCAT transgenic rabbits show a marked decrease in aortic atherosclerosis, although the converse is true for similarly transgenic mice. Unesterified cholesterol (UC) is esterified by LCAT in HDL2 and in HDL3, the much-preferred substrate. Regulation of hepatic transcription of the LCAT gene and regulation of LCAT activity are not well understood. However, vitamin E does not appear to affect human LCAT activity.

CE Transfer Protein

CETP, a 66- to 74-kDa hydrophobic glycoprotein, is expressed in liver, spleen, adipose tissue, kidney, and skeletal muscle (see Lipoproteins in Health and Disease, chapter 15). Its principal function is to exchange triglycerides (TGs) fromapoB-containing particles for CE from HDL particles. CETP is localized principally on the larger Lp(A-I) particles.

Increased expression, or activity, of CETP would predictably reduce HDL-C levels and particle size. However, the effects of variation in CETP activity remain controversial. Experimentally, atherosclerosis is increased in cholesterol-fed mice lacking apoE and LDL receptor (LDLR) genes and overexpressing human CETP and is reduced in naturally CETP-deficient mice of this type. However, Japanese patients genetically deficient in CETP are felt to have an increased incidence of atherosclerosis.

A common CETP intron gene polymorphism, B1/B1, is in linkage disequilibrium with a polymorphism in its promoter. It is found in 36% of patients with CAD and has 30% greater plasma CETP concentration, 13% lower HDL levels, and 3 times more rapid coronary luminal narrowing than its homozygous B2/B2 counterpart (16% of patients). Probufol, a powerful antioxidant drug, increases CETP activity. It has been shown to lower HDL2b by 53% and its apoA-I protein level by 67%, changes that are correlated with worsening femoral arterial obstruction, suggesting that increased CETP activity is atherogenic. However, genetically increased CETP has tended to be protective and reduced CETP has tended to be atherogenic in 2 populations at low cardiovascular risk (low LDL-C and high HDL-C) and with low prevalence of CAD. These apparently conflicting findings would be reconciled if CETP activity is either protective or harmful depending on the atherogenicity (remnants, apoC-III or apoE4 content, and size) of the apoB particles receiving the CE from HDL. As Figure 1 suggests, apoB particles that return to the liver contribute to reverse cholesterol transport; those preferred by the macrophage contribute to atherogenesis.

Regulation of CETP transcription is by the same LXR/RXR nuclear receptor heterodimer that activates transcription of ABCA1. Similarly, ARP-1 has been shown to upregulate CETP gene transcription.

Thus, a plausible candidate mechanism for the antioxidant vitamin–associated blunting of the favorable effects of lipid therapy on HDL2-C and coronary disease would be an antioxidant-mediated induction of CETP activity, as has been shown for probucol and vitamin E.

PL Transfer Protein

PLTP facilitates transfer of the surface lipids (PL and unesterified cholesterol) from the apoB-containing lipoproteins onto HDL. PLTP also directly mediates PL transfer among HDL particles, thus promoting HDL size heterogeneity. The PLTP-mediated conversion of HDL into larger and smaller particles is thought to involve displacement from the lipoprotein surface of small lipid-poor apoA-I–containing particles, causing parent particle instability with resulting fusion into larger HDL. This effect applies to Lp(A-I) and Lp(A-I, A-II) HDL particles. PLTP activity is strongly correlated with Lp(A-I) plasma concentration but not Lp(A-I, A-II) plasma concentration. However, PTLP activity is not increased by lipid therapy (niacin plus simvastatin), which substantially increases Lp(A-I) concentration, suggesting that the rise in Lp(A-I) is not mediated directly by PLTP. By extension, the antioxidant vitamin–mediated blunting of Lp(A-I) would not be related to PLTP.

HL/LPL/EL

HLs, LPLs, ELs, and pancreatic lipases (see Lipoproteins in Health and Disease, chapter 12) are members of a gene family involved in processing ~150 g dietary triglyceride daily. LPL is synthesized predominantly in adipose, heart, and skeletal muscle and is adherent to vascular endothelium of extrahepatic tissues; its activity is regulated and is tissue specific. HL is synthesized only in the liver, is bound to hepatic sinusoidal vascular endothelium, and functions only in the liver. The principal in vivo substrates of LPL and HL are TGs and PL. LPL requires apoC-II as a cofactor; HL has no required cofactors. LPL serves the first phase of lipolysis of VLDL and chylomicrons (Figure 1); it rapidly hydrolyzes core TGs, resulting in the shedding of the redundant surface from these particles as lipid-poor apoA-I and/or apoE PL-UC disks (Figure 1). These coalesce with larger HDL or acquire PL and UC from ABCA1 (see Figure 1). Lymph of VLDL- and chylomicron-remnant particles involves LPL and enters HDL3. Lipases influence HDL levels; among the general population, LPL is positively associated with HDL and HDL2 levels and is negatively associated with their catabolism, whereas HL has an inverse association that is particularly strong for HDL2-C levels and for LDL particle size.

Regulation of LPL and HL activity is under investigation. A growing body of evidence points to the modulation by activated LXR and RXR nuclear receptor heterodimers of the expression of certain of the genes regulating lipoprotein metabolism, including LPL and sterol regulatory element-binding protein-1.

Little is known about the roles and regulation of EL, which is of interest because of its high degree of phospholipase activity.
SR Class B Type I

SR-BI, an 82-kDa protein of the CD36 family, mediates selective uptake of HDL-C and CE in the liver, steroidogenic tissues, and macrophages. It also binds LDL at a separate receptor domain. SR-BI–independent mechanisms also appear to mediate selective CE uptake from HDL3, and in vitro, the rate of CE-selective uptake from donor HDL particles is proportional to the amount of CE initially present on particles, suggesting that the principal contributors to this transport process are the larger HDL2s. It is proposed that HDL cholesteryl, the principal source of biliary cholesteryl, is first taken up as a particle by SR-BI into the hepatocyte endosome system and that by selective endosome sorting, the cholesterol is removed, and the lipid-poor HDL is resecreted into plasma. In the liver, SR-BI, expressed on apical and basolateral plasma membranes, plays a key role in the uptake of HDL CE and its sorting, processing, and excretion in bile. In an in vitro system, the rate of CE-selective uptake from donor HDL particles is proportional to the amount of CE initially present on particles, suggesting that the principal contributors to this transport process are the larger HDL2s. It is proposed that HDL cholesteryl, the principal source of biliary cholesteryl, is first taken up as a particle by SR-BI into the hepatocyte endosome system and that by selective endosome sorting, the cholesterol is removed, and the lipid-poor HDL is resecreted into plasma.

SR-BI deficiency in mice selectively diminishes biliary cholesterol secretion without diminishing bile acid or PL secretion; SR-BI deficiency does not impair absorption of dietary cholesterol. Overexpression of SR-BI in liver reduces VLDL- and LDL-apoB as well as HDL levels, increases reverse cholesterol transport, and decreases susceptibility to atherosclerosis. Conversely, genetic downregulation of SR-BI activity in susceptible mice increases atherosclerosis. Probucol, a potent antioxidant, markedly increases SR-BI–mediated selective HDL CE uptake in intact mouse liver and in SR-BI–expressing hamster ovary cells in culture. This occurs without increased SR-BI membrane protein, apparently by a size-independent alteration of the probucol-carrying HDL that enhances the HDL–SR-BI interaction.

Regulation of SR-BI expression is under active investigation. PPAR activators acting through RXR heterodimers have substantially increased the expression of SR-BI in differentiated human macrophages and in apoE-null mouse atherosclerotic plaque. PPARα and PPARγ are expressed in such macrophages, as is the human analogue of murine SR-BI. By extrapolation, increased expression of SR-BI with ligands for PPARs and possibly for RXRs should reduce LDL-C and HDL-C, accelerate reverse cholesterol transport, and decrease atherosclerosis. This is consistent with early clinical experience, except that HDL-C is modestly increased, suggesting other loci of gene regulation by these ligands in vivo.

Megalin/Cubulin

Among the largest plasma membrane proteins yet described, these 2 are colocalized on the apical brush border of renal tubular cells and ileal enterocytes. Megalin (LDLR-related protein [LRP]-2) is an ~500-kDa member of the LRP family, which is thought to depend on cooperation with membrane-anchored cell surface receptors to promote LRP-mediated endocytosis of the ligand. Cubulin is a 460-kDa membrane surface receptor that binds the smallest HDL particles that normally appear in the glomerular filtrate, particles <8 nm in diameter, which constitute up to 8% of plasma apoA-I. These lipid-poor HDLs are shown in Figure 1 as acceptors of UC and PL from ABCA1 and are shown in Figure 2 as HDL3c. Once the megalin-cubulin-HDL3c complex is internalized, the ligand is separated from the receptor, which is recycled to the cell surface while HDL undergoes lysosomal degradation. The expression of both receptors in vitro is increased by RA, but not by sterol depletion. Increased renal catabolism of HDL3c by upregulation of this receptor complex could deplete this precursor of HDL3 and HDL2 and, thus, may plausibly explain the HDL2-lowering effects of our antioxidant cocktail. However, the rate-limiting factor for this mechanism of HDL catabolism is more likely to be the plasma concentration of HDL3c, the principal determinant of its appearance in the glomerular filtrate. Therefore, we doubt that the antioxidant effect in question is mediated by enhanced megalin/cubulin expression.

In the End, the Chemistry Just Wasn’t Right

We have attempted to explain why adding an antioxidant vitamin cocktail to LDL-lowering and HDL-raising therapy blunts the expected increase in HDL2-C, Lp(A-I), and HDL particle size while appearing to diminish the antiatherogenic and favorable clinical effects of lipid therapy. To do so, we have reviewed current understanding of the actions and regulation of individual components of the reverse cholesterol transport pathways.

From the present review, one would predict that selective downregulation of the expression of apoA-I, ABCA1, LCAT, and SR-BI genes would blunt reverse cholesterol transport and, except for SR-BI, decrease HDL2-C. Thus, diminished apoA-I, ABCA1, and LCAT gene expression appear to be potential candidates to explain the antioxidant effects. However, LCAT activity has not been shown to be altered by vitamin E.

The effects of increasing CETP activity on reverse transport are not so easily predictable. CETP-mediated transfer of CE to apoB-containing particles may prove to be proatherogenic, particularly if the LDLRs are downregulated and the recipient particles contain atherogenic apolipoproteins (eg, apoC-III and apoE4/E4; see Figure 1). Consistent with this, we have the clinical result that a powerful antioxidant, probucol, increases measured CETP activity, strikingly decreases HDL-C and HDL2-C, and, if anything, promotes atherogenesis. Vitamin E has been shown to have similar effects on CETP. Thus, CETP activation appears to be a prime suspect in the antioxidant effects observed in HATS.

Antioxidant-mediated upregulation of SR-BI expression or enhancement of its HDL interaction would reduce HDL2-C levels but should also lower LDL-C and favorably effect atherogenesis. For these reasons, increased SR-BI expression appears to be an unlikely candidate to explain the antioxidant effect in question.

Downregulation of hepatic lipase activity by intensive lipid therapy results in increased HDL2-C and decreased atherosclerosis, apparently via an LDL-size effect. A blunting of this response by antioxidants would simulate the findings in HATS; thus, upregulation of HL activity by antioxidants is another candidate mechanism. However, we lack sufficient understanding of HL regulation to make speculations.

Although human LPL activity is correlated with HDL2-C levels and inversely with HDL catabolism and although LPL activity may be modulated by oxysterol-activated LXR nu-
clear receptors, the lack of effect of our antioxidants on the TG response to simvastatin-niacin (see Table 2) suggests that the antioxidant effect in question is not mediated by changes in LPL.

By the above reasoning, the possible culprits for the antioxidant effect in question may be boiled down to 3: (1) decreased hepatic or intestinal apoA-I synthesis (or its increased catabolism), (2) decreased macrophage ABCA1 expression, and/or (3) increased CETP activity. The latter has been confirmed for vitamin E.

A common thread among these mechanisms is the role played by oxysterol-activated LXR, the 9-cis-RA–activated RXR, and (to a lesser extent) the RA-activated RAR nuclear receptors in regulating the cell-specific expression of each of these genes. LXR nuclear receptors also regulate the transcription of several other genes responsible for cholesterol metabolism, including LPL, ABCG1, ABCG5, ABCG8, apoE, and CYP7A1. One could postulate that fat-soluble antioxidant vitamins, entering cells on endocytosed LDL or HDL, might diminish oxysterol/oxidative stress–related activation of LXR or that RA (from β-carotene) might competitively interfere with 9-cis-RA activation of RXR, both resulting in the diminished expression of apoA-I and/or ABCA1. Alternatively, by decreasing the intestinal concentration of oxidized LDL, these vitamins may indirectly down-regulate the LXRe-responsive ABCA1 gene. Or, simultaneous hepatic upregulation of CETP expression and downregulation of apoA-I synthesis, mediated by a putative antioxidant activation of ARP-1, could cause the HDL2-C effects under consideration.

Can We Still Be Friends?

Should antioxidant vitamins be combined with lipid therapy under any circumstances? The large prospective trials (see Table 1) do not demonstrate any benefit from individual antioxidant vitamins given without lipid therapy. However, might there be a favorable interaction between these 2 treatments, enhancing the benefits of lipid therapy? Our HATS trial showed no significant benefit from the vitamin cocktail given alone; furthermore, the combined therapies interacted adversely, actually detracting from the benefits of lipid therapy. The recently published Heart Protection Study, which compared antioxidant vitamins versus their placebo in a factorial design with simvastatin (40 mg QD) versus its placebo, showed no cardiovascular (or nonvascular) benefit from a combination of vitamins similar to the combinations in HATS. Indeed, there were small but highly significant in-treatment differences between the antioxidant vitamin and antioxidant placebo groups in LDL-C, TG, and HDL-C levels (P<0.0001), predictably favoring placebo, and there was a nonsignificant trend (P=0.3) toward a 5% cardiovascular mortality increase (see Table 1).

Thus, the totality of evidence for currently studied antioxidant vitamins demonstrates that they are of no benefit to the general population at high or at low cardiovascular risk when taken for the prevention of native vascular disease (or cancer); other proposed special indications require further prospective proof of benefit. Indeed, by creating a diversion from proven therapies, the use of these antioxidants may actually do harm. Furthermore, our HATS trial, if confirmed, raises the important caveat that therapies designed to substantially raise HDL2 should not be combined with antioxidant vitamins. It is important to distinguish the current negative results of antioxidant trials from the prevailing view that oxidation of LDL contributes importantly to atherogenesis. Eventually, more powerful, appropriately targeted, or less HDL-2 adverse antioxidants may be proven to be protective.

We conclude that by analogy, the farmer and the cowgirl (antioxidants and lipid drugs) could be friends, but at best, the relationship would be platonic, and it could get nasty.

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