A comprehensive approach to the treatment of atherosclerosis by increased ApoA-I and HDL-cholesterol levels

Dr. Amitabha Kar

Abstract
In human body, cholesterol is transported around in the form of lipoprotein (lipid/protein) complexes, because it is almost insoluble in water. Extensive study shows that High-density lipoprotein (HDL) particles transport cholesterol from tissues back to the liver for excretion. Epidemiological studies have shown an inverse relationship between blood levels of HDL-cholesterol (HDL-c) and the incidence of clinically significant atherosclerosis. The beneficial effects of HDL in altering atherosclerotic disease are believed to involve elevated levels of HDL enhancing the efflux of cholesterol from arterial walls, increasing transport of cholesterol from arteries to the liver for excretion. This reverse cholesterol transport (RCT) pathway is used to explain both HDL’s role in lipid metabolism and the inverse association between HDL-c plasma concentration and the risk of cardiovascular disease. Based on the RCT model, ApoA-I is an attractive target for therapeutic intervention. Experimental manipulations to increase production of ApoA-I have been associated with reduced atherogenicity. There is a continuing need for novel therapies that increase the biosynthesis of HDL, to inhibit the progression of and even bring about regression of atherosclerosis. Small molecule compounds that increase the production of endogenous ApoA-I would be attractive therapeutic agents for treating dyslipidemias.

Keywords: Cholesterol, apolipoprotein A-I (ApoA-I), high-density lipoprotein (HDL), reverse cholesterol transport (RCT), atherosclerosis, dyslipidemia

Introduction
Because of insoluble properties in water, cholesterol is almost transported around the body in the form of lipoprotein (lipid/protein) complexes [Fig-1].

The liver produces very low density lipoproteins (VLDL) and secretes them into plasma, where they are converted to low-density lipoprotein (LDL) particles and non-esterified fatty acids [1]. High-density lipoprotein (HDL) particles transport cholesterol from tissues back to the liver for excretion, as fecal sterols and bile acids. HDL exists primarily in two forms, one containing apolipoprotein A-I (ApoA-I) and apolipoprotein A-II (ApoA-II), and one containing ApoA-I alone [2]. The cardio-protective effect is largely due to ApoA-I.
In addition to their role in RCT, HDL particles comprises anti-inflammatory, anti-oxidative, anti-apoptotic, anti-thrombotic, vasodilatory, and antiinfective properties. Statistics of lipid metabolism also reveals that dyslipidemia is associated to atherosclerosis [3A, 3B, 3C, 3D, 3E]. The validity of the hypothesis was ultimately established by major epidemiological studies, such as the “Seven Countries Study,” which, with a 25-year follow-up, found that across cultures, cholesterol levels were linearly related to coronary heart disease mortality [4]. Other epidemiological studies, including the Framingham and Procam studies, and it has also found that, there is an inverse relationship between blood levels of HDL-c and the incidence of clinically significant atherosclerosis [5]. This inverse association between HDL-c concentrations and cardiovascular risk is apparently continuous; there seems to be no threshold value. In fact, each 1 mg/dL increment in serum HDL-c is associated with a 2%–3% decrement in cardiovascular risk, while a 1% reduction in LDL-c decreased the risk of coronary heart disease (CHD) by 1%–2% [6]. The Veterans Affairs HDL-c Intervention Trial (VA-HIT) examined the benefit of secondary prevention in patients with low HDL-c levels and a history of coronary artery disease (CAD). Apolipoprotein B is the primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL particles (LDL-known commonly by the misnomer “bad cholesterol” when in reference to both heart disease and vascular disease in general), which is responsible for carrying fat molecules (lipids), including cholesterol, around the body (within the water outside cells) to all cells within all tissues. While all the functional roles of ApoB within the LDL (and all larger) particles remains somewhat unclear, it is the primary organizing protein (of the entire complex shell enclosing/carrying fat molecules within) component of the particles and is absolutely required for the formation of these particles. What is also clear is that the ApoB on the LDL particle acts as a ligand for LDL receptors in various cells throughout the body.

<table>
<thead>
<tr>
<th>Metabolic Syndrome: Disease of the Modern Era</th>
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<tbody>
<tr>
<td>Constellation of several risk factors that increase chance of coronary artery disease, peripheral vascular disease, stroke and type 2 diabetes.</td>
</tr>
<tr>
<td>Combination of 3 or more of the following risks:</td>
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<tr>
<td>· Abdominal obesity</td>
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<td>· Triglyceride levels above 150 mg/dL</td>
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<td>· Low HDL cholesterol</td>
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<td>· Elevated blood pressure (&gt;130/85 mm Hg)</td>
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<td>· Fasting blood glucose &gt; 100 mg/dL</td>
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<td><em>Aging a major contributor: prevalence in 20-29 yr olds = 6.7%; 60-64 yr olds = 43.5%</em></td>
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Fig 2: demonstrates several risk factors associated with CAD

In patients with existing CAD, the only lipid abnormality seems a low HDL-c level. The INTERHEART study, an international case control study comparing myocardial infarction survivors with age-and gender-matched controls, showed the ApoA-I/Apo-B ratio to be the strongest modifiable protective factor [7].

Reverse Cholesterol Transport (RCT)
The process whereby excess cholesterol in peripheral cells, especially foam cells, is returned to the liver for degradation and excretion.

Fig 3: demonstration about the different states of Lipoproteins.
Fig 4: demonstrates about the site of synthesis of Lipoproteins.

Fig 5: Major Apolipoproteins and their functions

<table>
<thead>
<tr>
<th>Apo</th>
<th>Lipo</th>
<th>Origin</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>ApoA-I</td>
<td>HDL</td>
<td>Liver, intestine</td>
<td>Activate LCAT, Cholesterol efflux via ABCA1 transporter</td>
</tr>
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<td>ApoB-100</td>
<td>VLDL, LDL</td>
<td>Liver</td>
<td>Ligand LDL receptor, TG transport from cells</td>
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<td>Apo(a)</td>
<td>Lp(a)</td>
<td>Liver</td>
<td>Inhibits fibrinolysis</td>
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<tr>
<td>ApoCII</td>
<td>HDL, VLDL</td>
<td>Liver</td>
<td>Activates lipoprotein lipase</td>
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<tr>
<td>ApoE</td>
<td>VLDL, IDL</td>
<td>Liver, intestine</td>
<td>Ligand, LDL receptor, LRP receptor</td>
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LCAT: lecithin:cholesterol acyltransferase
ABCA1: ATP binding cassette protein A1
LRP: LDL receptor related protein

RCT involves apoA-I [Fig:5], ABCA1 and LCAT as well as Receptors on the liver for uptake of the excess cholesterol. While the mechanisms of the beneficial effects of HDL in altering atherosclerotic disease are not completely understood, it is believed that elevated levels of HDL enhance the efflux of cholesterol from arterial walls, increasing transport of cholesterol from arteries to the liver for excretion. Elevation of ApoA-I increases paraoxonase activity, and enhances anticoagulant and anti-inflammatory activities. HDL also promotes fibrinolysis. Additionally, HDL particles protect against LDL oxidation, plays a key step in promoting cholesterol uptake by arterial macrophages. The major steps in the reverse cholesterol transport (RCT) pathway [Fig:6] are the active efflux of cholesterol and phospholipids from cells by ATP-binding cassette transporter A1 (ABCA1), the binding of cholesterol to apolipoproteins, forming pre-HDL, the esterification of HDL-bound cholesterol by lecithin cholesterol acyltransferase (LCAT); the resulting cholesteryl esters (CEs) are the core lipids of HDL [Fig:7], CETP-mediated exchange of CEs and TGs between HDL and Apo B-containing particles, and hepatic lipase (HL)-mediated uptake of cholesterol and TGs by the liver. This RCT concept is used to explain both HDL’s role in lipid metabolism and the
inverse association between HDL-c plasma concentrations and cardiovascular disease risk. The effects of mutations in the various proteins and enzymes of the RCT pathway have helped in our understanding of cholesterol metabolism. Based on the RCT model, ApoA-I is an attractive target for therapeutic intervention. Experimental manipulations that increase production of ApoA-I have been associated with reduced atherogenicity. Human ApoA-I is protective in transgenic animal models\[8A, 8B, 8C, 8D\] and infusion of ApoA-I prevents atherosclerotic lesions and leads to regression of atherosclerotic plaques in human patients. Small-molecule compounds that increase the production of endogenous ApoA-I would be attractive therapeutic agents for treating dyslipidemias.

**Fig 6:** demonstrates details about Reverse Cholesterol Transport System

**Fig 7:** demonstrates Lecithin, Cholesterol Acyl Transferase (LCAT)

**Familial HDL Abnormalities**

As familial HDL-c deficiency is often associated with family histories of premature CAD, much effort has been directed to understanding the molecular defect(s) involved there with \[9A, 9B, 9C\]. Increased HDL-c concentrations are generally accepted to be protective against the development of atherosclerosis and CAD, but studies have suggested that the underlying cause of the increased HDL-c may be important in whether it is protective. The familial hypoalpha-lipoproteinemia are rare lipoprotein disorders characterized by low levels of plasma HDL\[Fig 8\]. However, despite of markedly reduced HDL levels, several of these
conditions, including Tangier disease, fish eye disease, and LCAT deficiency, are not associated with premature atherosclerosis. Moreover, some mutations in LCAT are known that result in increased CAD\textsuperscript{[10A, 10B]}. Mutations leading to reduced CETP activity result in less CE being directed into Apo-B-containing particles (VLDL and LDL) and more remaining in HDL, resulting in increased HDL-c concentrations. Mutations leading to reduced hepatic lipase (HL) activity are rare and are associated with increased HDL-c concentrations and CAD.

The ATP-binding cassette transporter A1 (ABCA1) protein regulates the efflux of cholesterol and phospholipids from the cell to apolipoprotein acceptors, the rate-limiting step in the removal of cellular cholesterol. Tangier disease is characterized by mutations in the ABCA1 gene, resulting in a defective ABCA1 protein and accumulation of cellular cholesterol, reduced plasma HDL-c, and increased risk for CAD; over 100 ABCA1 coding variants are known\textsuperscript{[11A, 11B, 11C, 11D]}. The plasma in case of Tangier disease (TD) patients has essentially no ApoA-I-containing lipoprotein (LpA-I), which in normolipidemic plasma is the majority of HDL. Residual amounts of ApoA-I in TD plasma have electrophoretic pre1-LpA-I mobility.

CETP deficiency causes hyper-alpha-lipoproteinemia, that is, marked elevation of plasma HDL-c\textsuperscript{[12]}. While the extent to which CETP deficiency is actually associated with protection against CAD is unclear, it gave rise to the idea of inhibiting CETP as a therapeutic strategy\textsuperscript{[13A, 13B]}. Extensive studies showed that, aortic atherosclerosis was established over 17 weeks in rabbits by balloon denudation and cholesterol feeding. Animals then received oral atorvastatin (5 mg/kg for 5 days) or two infusions of HDL particles (8 mg/kg ApoA-I) 2 days apart. Lesion size and composition were then assessed. HDL, but not atorvastatin, reduced lesion size by 36\%\textsuperscript{[14]}. In patients with heterozygous familial hypercholesterolemia, a single infusion with recombinant pro ApoA-I particles (precursor of ApoA-I) resulted in a 34\% sustained 10-day increase in cholesterol excretion, with a net removal of 5\%–7\% of total body cholesterol. Fecal sterol excretion was measured for 9 days before and 9 days after an intravenous infusion of the pro-ApoA-I liposome complexes. Plasma ApoA-I and HDL-c levels increased transiently during the first 24 h. Fecal excretion of cholesterol (neutral sterols and bile acids) increased in all subjects. Control infusions with only liposomes in two of the patients did not affect cholesterol excretion\textsuperscript{[15]}. Another line of research is the use of peptides that mimic ApoA-I. ApoA-I is a large protein, comprising 243 amino acids, making its recombinant preparation difficult and expensive. Additionally, intravenous administration is necessary, which is inconvenient. Efforts have been made at finding peptide mimetics that produce similar results to ApoA-I, but that would be easier to manufacture and administer\textsuperscript{[16]}

**Primary (familial) Lecithin**

Cholesterol acyltransferase (LCAT) deficiency is a genetic disease associated with corneal opacity, anemia, and proteinuria with renal failure. It is caused by the complete or near absence of LCAT activity. ApoA-I is similarly characterized by a cysteine-for-arginine substitution, at position 151(R151C). These cysteine-for-arginine substitutions allow disulfide-linked dimers to form. The mutations appear to make the ApoA-I and ApoA-I proteins functionally more effective than normal ApoA-I. In several studies it has been found that, HDL formulations of recombinant ApoA-I-phospholipid complexes brought about rapid regression of a focal carotid atheroma and protection from myocardial infarction\textsuperscript{[14A, 14B]}. Extensive studies showed that, aortic atherosclerosis was established over 17 weeks in rabbits by balloon denudation and cholesterol feeding. Animals then received oral atorvastatin (5 mg/kg for 5 days) or two infusions of HDL particles (8 mg/kg ApoA-I) 2 days apart. Lesion size and composition were then assessed. HDL, but not atorvastatin, reduced lesion size by 36\%\textsuperscript{[14]}. In patients with heterozygous familial hypercholesterolemia, a single infusion with recombinant pro ApoA-I particles (precursor of ApoA-I) resulted in a 34\% sustained 10-day increase in cholesterol excretion, with a net removal of 5\%–7\% of total body cholesterol. Fecal sterol excretion was measured for 9 days before and 9 days after an intravenous infusion of the pro-ApoA-I liposome complexes. Plasma ApoA-I and HDL-c levels increased transiently during the first 24 h. Fecal excretion of cholesterol (neutral sterols and bile acids) increased in all subjects. Control infusions with only liposomes in two of the patients did not affect cholesterol excretion\textsuperscript{[15]}. Another line of research is the use of peptides that mimic ApoA-I. ApoA-I is a large protein, comprising 243 amino acids, making its recombinant preparation difficult and expensive. Additionally, intravenous administration is necessary, which is inconvenient. Efforts have been made at finding peptide mimetics that produce similar results to ApoA-I, but that would be easier to manufacture and administer\textsuperscript{[16]}

**Life style modification**

Much research suggests that the first step in increasing HDL-c levels should be life style modification. Regular aerobic exercise, loss of excess weight (fat), cessation of cigarette smoking, and changes in diet can all increase HDL-c levels\textsuperscript{[Ashen and Blumenthal, 2005; Kodama et al. 2007; Mooradian et al. 2006; Dullens et al. 2007]}. Dietary soy protein, soluble fiber, and plant sterol/ ester-containing

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Fig 8: demonstrates HDL Protective Role in Human body
margarines have all been shown to favorably reduces the LDL: HDL ratio (Hermansen et al. 2003). Diets rich in whole grain foods tend to increase serum HDL-c levels, while decreasing serum LDL-c, triacylglycerol levels, and blood pressure (Anderson, 2003). The effects of soy isoflavones are somewhat controversial (Dullens et al. 2007). Polynphenols have become a focus of research for their role in the prevention of cardiovascular disease and ability to raise HDL-c. Polynphenols are common components of the human diet, present in many foods and beverages of plant origin, including tea, wine, cocoa, and soy. Epidemiological studies have repeatedly shown an inverse association between the risk of myocardial infarction and the consumption of tea or wine and intake levels of some particular flavonoids, but no clear association has been found in clinical studies with primary clinical endpoints (Scalbert et al. 2005; Manach et al. 2005). In fact, in twelve intervention studies with differing polyphenol sources, six showed no effect on lipid parameters and six showed an improvement (Manach et al. 2005). Such inconclusive data has clouded the use of polyphenols (Zern and Fernandez, 2005). Moderate consumption of red wine (one or two drinks per day) has consistently been associated with a reduced risk of cardiovascular disease, a phenomenon to explain the so-called “French Paradox,” the relatively low rate of vascular disease in the French population, despite a high level of saturated fats in their diet (Poussier et al. 2005; Zern and Fernandez, 2005; Renaud and de Lorgeil, 1992; Burr, 1995; Schäfer et al. 2007). The polyphenol resveratrol (trans-3,5,4'-trihydroxystilbene), a stilbenoid antioxidant found in the skin of red grapes, wine, peanuts, and some berries, has been shown to exhibit cardio-protective properties (Wang et al. 2005). Thus, mild-to-moderate alcohol consumption appears to be reasonable for many people; in contrast hepatic dysfunction and addiction may outweigh the benefits. Improvement in HDL-c with lifestyle changes may often be associated; however, the interaction between genes and the environment may influence the magnitude of any improvement. Thus, when lifestyle modifications are insufficient, medications are used. Approaches to raise anti-atherogenic HDL-c have attracted much attention, because of expanding disease populations with low levels of HDL-c or high cholesterol, such as patients with type 2 diabetes, metabolic syndrome, dyslipidemia, and menopause. To date, several medications that have been approved that increase HDL-c and new approaches are being sought. Niacin is the most effective drug currently available to raise HDL-c levels. At higher pharmacological doses, niacin can reduce LDL-c, triglycerides [Fig:9, Fig:10], and apo-B, while increasing HDL-c and ApoA-I (Chapman, 2005). These beneficial lipoprotein changes have been shown to translate for benefits, decreasing morbidity and mortality from cardiovascular causes (McCormack and Keating, 2005). Nicotinic acid, as vitamin B3, is available without prescription; however, side effects tend to be frequent when taken under suboptimal conditions. Extended- or prolonged-release niacin (Niaspan), marketed by Kos Pharmaceuticals, decreases flushing, niacin’s most common side effect and increased HDL-c by ~25% from baseline at 200 mg/kg in clinical studies (Pal and Pillarisetti, 2007; McCormack and Keating, 2005; Birjmoohan et al. 2004). With the discovery of the niacin receptor (HM74a, PUMA-G), several drug companies are working on developing novel agonists (Pal and Pillarisetti, 2007).
27% increase in HDL Cholesterol (Forrester and Shah, 2006). This drug is currently approved in many markets under the brand name Acomplia. However, in June 2007, the U.S. FDA’s Endocrine and Metabolic Drugs Advisory Committee did not recommend approval of this strategy for weight management.

**CETP inhibitors**

Cholesterol ester transfer protein (CETP) is a glycoprotein that facilitates the transfer of cholesteryl esters from HDL-c to Apo B-containing lipoproteins in exchange for triglycerides (Pal and Pillarisetti, 2007). Subjects with CETP deficiencies, because of CETP gene defects, have elevated plasma levels of HDL-c and ApoA-I. However, the anti-atherogenic role of CETP is complex because, despite raising HDL-c levels, it also slows the metabolism of HDL-c (Rader, 2006, 2007a). To date, two CETP inhibitors, JTT-705 and torcetrapib, have been evaluated clinically and have shown efficacy of increase HDL-c (Rader, 2006, 2007a; Barter et al. 2007). In early clinical studies, torcetrapib showed a pronounced effect on plasma lipoproteins in patients with low HDL-c levels, and reduced the levels of LDL-c and apolipoprotein B, both as a monotherapy and in combination with atorvastatin (Rader, 2006, 2007a). After the phase II dose-ranging trials, torcetrapib, despite being associated with substantial increase in HDL-c and decrease in LDL cholesterol, was also associated with an increase in blood pressure, and no decrease in the progression of coronary atherosclerosis (Nissen et al. 2007b). The lack of sufficiency may be related to the mechanism of action of this drug class or to molecule-specific adverse effects (Rader, 2007b; Barter et al. 2007).

After demonstrating in phase II, no additional data has yet been published on JTT-705. Inflammation has a fundamental role in mediating all stages of atherosclerotic disease. AGI-1067 (probucol monosuccinate, succinobucol) is being assessed for the treatment of restenosis and possibly atherosclerosis. The pharmacological activities of AGI-1067 are the ability to block the expression of oxidation-sensitive inflammatory genes, including genes that encode vascular cell adhesion molecule-1 and monocyte chemotactic protein-1.

**Phospholipids**

Phosphatidyl-inositol can stimulate reverse cholesterol transport by enhancing the flux of cholesterol into HDL and by promoting the transport of HDL-c to the liver and bile. Currently, phosphatidyl-inositol (PI) is being evaluated in human subjects. At doses as high as 5.6 g/day, PI demonstrated raise of HDL-c of 18% over a 2-week period, and a fall in triglycerides by 36% in fed subjects. This product is in clinical development (Burgess et al. 2005). CSL-111 is a reconstituted HDL, consisting of ApoA-I from human plasma combined with soybean phosphatidylcholine, that resembles native HDL (Tardiff et al. 2007b). In a 183-patient phase II clinical trial, there was no significant reduction in atheroma or plaque volume, compared with the placebo, but there was a statistically significant improvement in the plaque characterization index and coronary score on quantitative coronary angiography [13].

**Conclusions**

The lipid hypothesis, now more than 100 years old, is based on the idea that dyslipidemia is central to atherosclerosis. Its validity was ultimately understood by major epidemiological studies, demonstrating that cholesterol levels were linearly related to coronary heart disease mortality. Further epidemiological studies showed an inverse relationship between blood levels of HDL-c and the incidence of clinically significant atherosclerosis; it appears that each 1 mg/dL increment in serum HDL-c is associated with a 2%–3% decrement in cardiovascular risk (Boden, 2000). While our understanding of the mechanism(s) by which HDL alters atherosclerotic disease remains incomplete, it is believed that elevated levels of HDL increase the transport of cholesterol from arteries and tissues to the liver for excretion. Based on the RCT model (Fig. 1), ApoA-I is an attractive target for therapeutic intervention. Experimental manipulations to increase ApoA-I have been shown to reduce atherogenicity. Human ApoA-I is protective in transgenic animal models (Shah et al. 1998; Rubin et al. 1991) and infusion of ApoA-I prevents atherosclerotic lesions and leads to regression of atherosclerotic plaques in human patients. There is an ongoing need for novel therapies to increase the biosynthesis of HDL, to inhibit the progression of and even bring about regression of atherosclerosis. The development of a small molecule drug that increased endogenous ApoA-I would be a major advance in treating lipid-related cardiovascular disease. Targeting the patient’s liver and small intestine to increase the transcription, synthesis, and secretion of ApoA-I is generally considered a promising approach for raising HDL-c levels. Currently, our ability to raise HDL is limited; lifestyle, including dietary, changes, niacin, fibrates, estrogen, and to some degree, the statins do raise HDL, but not very potently. Several new drug classes are under investigation in this very active field.

**Authors’ contribution**

Author has extended in depth research and exclusive study regarding the present spectrum that has been manifested to write him this review in favour of mankind and well-being of health science and cultivation.

In this paper, the author achieved extreme guidance favoring the in depth cultivation with a positive output from Partha Majumder, Biomedical Scientist and Systems Biologist, Former Principal Scientist (Helixin Biosystems) and Former Head & Coordinator, Department of Applied Biotechnology and Bioinformatics, Sikkim Manipal University(CC:1637), Kolkata, India.

**Acknowledgement**

It is an established fact that every mission needs a spirit of dedication and hard work but more than anything else it needs proper guidance. In fact, author feels proud in taking this opportunity to express his heartiest regards and deep sense of gratitude to his beloved wife Mrs. Ranita Kar for her evergreen inspiration as well as spirit for progress, research and all kinds of pursuit of excellence in author’s life and achievements.

**References**


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