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Implications of reverse cholesterol transport: Recent studies

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ABSTRACT

Introduction: There is a strong epidemiological relationship between high density lipoproteins and atherosclerotic coronary vascular disease (ASCVD). The process of reverse cholesterol transport (RCT) has been hypothesized to help explain this relationship. The corollary that raising HDL should reduce ASCVD is also drawn from this relationship. In recent years, the metabolism of HDL has become better understood. A hypothetical process for explaining RCT has been superimposed on the currently understood HDL metabolic pathways.
Methods: Outline of HDL metabolism and the superimposed RCT process. Literature review of studies of persons with genetic defects, HDL cholesterol raising clinical trials, Mendelian randomization studies and treatments with molecules that mimic HDL.
Conclusions: Mutation studies of ABCA1, LCAT and SR-B1 genes in humans showed expected variations in HDLC but little association with ASCVD and there was no significant association between HDLC and ASCVD in Mendelian randomization studies. Elevations in HDLC due to treatment with niacin and cholesteryl ester transport protein inhibitors in randomized trials raised HDLC but did not significantly reduce risk of ASCVD. Treatment with molecules that mimic HDL did not seem to reduce ASCVD. Thus, recent evidence does not seem to support RCT as currently proposed. This hypothesis seems to need substantial revision.

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Contents

1. Introduction

The lipid hypothesis that cholesterol (C) deposition is a major cause of atherosclerotic cardiovascular disease (ASCVD) dates back about 100 years ago to Anitschkow's work with rabbits [1]. In the 1950s, Gofman and associates showed that lipoproteins can be fractionated and showed that all cholesterol carrying particles were not the same but that low density lipoproteins (LDL) were more atherogenic

Abbreviations: C, Cholesterol; ASCVD or CAD (older term), coronary artery disease, atherosclerotic coronary vascular disease; LDL, low density lipoprotein; HDL, high density lipoprotein; RCT, reverse cholesterol transport; VLDL, very low density lioprotein; IDL, intermediate density lipoprotein; apo, apolipoprotein; ABC, ATP-binding cassette transporter; SR, scavanger receptor; B1, class B type I; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transport protein; CIMT, carotid intima-media thickness; MI, myocardial infarction; PLTP, phospholipid transport protein; (HL), Hepatic lipase.

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than other lipoproteins [1]. Moreover, they fractionated high density lipoprotein (HDL) into subclasses of larger, more-buoyant HDL_2 (d. 1.063–1.125 g/mL) and smaller, more-dense HDL_3 (d. 1.125–1.21 g/mL) [2].

In the 1970s and 80s Goldman and Brown [3] demonstrated that LDL was taken up by receptor mediated endocytosis such that persons with a heterozygous LDL receptor deficiency and very elevated levels of LDL were at very high risk for developing CAD with homozygotes dying of heart disease at an early age unless they received a liver transplant. The importance of this work was recognized by the Nobel Prize in 1985.

Much research has been devoted towards finding lipid altering therapies to reduce CAD. In 1970, the National Institutes of Health began an epic undertaking that would end in a large-scale, long-term, double-blind study which was completed in 1984. This study demonstrated that lowering LDLC by treatment of hypercholesterolemic men with the bile acid binding resin cholestyramine reduced major CAD events by 19%, with a statistically significant p value of less than 0.05 [4,5]. In 1976, Dr. Akira Endo began a extraordinary project [6] that would ultimately lead to the development and use of 3-hydroxymethylglutarate reductase inhibitors (statins) by the 1990s.

Merck's lovastatin was approved by the Federal Drug Administration in 1987. A series of randomized clinical trials culminating in the Scandinavian Simvastatin Survival Study published in 1994 showed that lowering LDLC reduced not only ASCVD mortality but all-cause mortality as well [7]. Furthermore, large scale studies indicated that statin treatment benefits men and women, old and young, and diabetics as well as nondiabetics [6]. These findings were proof of principle for the LDL lipid hypothesis that elevated LDLC was an important contributor to atherosclerosis.

Over 50 years ago, based on biochemical evidence, Glomset, Wright, and Ross proposed that HDL are the major species transporting cholesterol from peripheral tissue to the liver for excretion, so-called reverse cholesterol transport (RCT) [2,8,9]. In the 1970s, this proposal was strongly supported by epidemiological studies that showed HDLC is a strong and independent inverse risk factor for ASCVD. It is estimated that a 10 mg/L increase in HDLC is associated with a 2–4% lowering of coronary death independent of LDLC [10,11], and this effect is even stronger than the estimated 0.5% drop in risk per 10 mg/L decrease for LDLC [6]. This strong epidemiological relationship between ASCVD and HDLC not only strengthened the concept of RCT but also the corollary to this hypothesis that raising HDLC by therapeutic intervention would reduce ASCVD.

An early event in atherosclerosis is the formation of lipid laden macrophages — called foam cells. It has been thought that RCT is especially important in removing cholesterol from macrophages and biomarkers that could reflect this process would be ideal instruments for identifying risk. It was hypothesized that therapies which would increase the flux of cholesterol from macrophages to HDL would reduce ASCVD [12,13].

A great deal has been learned about the metabolism of LDL. As shown in Fig. 1, first, very low density lipoprotein (VLDL) is converted to intermediate density lipoprotein (IDL) by hepatic lipase (HL) in the liver. IDL are remnant lipoproteins, rich in apolipoprotein (apo) E that are rapidly removed from blood. Much of the IDL is taken up by the apo E-apo B receptor (also known as the LDL receptor), and IDL not removed by the receptor becomes LDL which are richer in cholesterol and contain mainly apo B-100 [3,4,6,14]. These are removed more slowly by the LDL receptor. LDL migrate in the beta-region upon electrophoresis and are considered atherogenic [15].

Although the inverse relationship between HDLC concentration and atherosclerosis has long been known, the metabolism of HDL was still poorly understood when the first LDLC lowering clinical trials were being conducted. It is only over the past 25 years that much has been learned about its complex extracellular metabolism. Based on this new information, the process of RCT seemed to fit well within these HDL metabolic processes and it was suggested that altering these processes to increase HDLC concentrations and/or the flux to HDL [12] would reduce ASCVD. Some studies examined genetic defects that based on the current RCT hypothesis and the newly understood metabolism of HDL should have led to an increase in ASCVD in affected persons. Other studies examined the corollary that therapeutic increases in HDLC should decrease atherosclerosis in treated persons. Here, we discuss these recent genetic findings and therapeutic trials which do not seem to support the concept of RCT as currently understood.

This Review discusses the following questions.

1. Does RCT truly proceed through the HDL metabolic pathways as currently understood?
2. Can one explain what appears to be a disparity between the results of current genetic and therapeutic clinical trials and the epidemiological evidence?
3. Might the idea of measuring a flux from tissue to HDL better reflect the process of atherosclerosis than measurement of HDLC concentrations per se?
4. If data drawn from our understanding of HDL metabolism does not support RCT, might the concept need extensive revision?

1.1. HDL structure, metabolic pathways and the proposed mechanism of RCT

The major proteins comprising HDL, along with site of synthesis, and known functions are shown in Table 1. Apo A-I is the major protein in HDL, consisting of about 70% of the total protein. Apo A-II comprises about 20–25% of the protein. There are minor proteins, of which apo E comprises the largest concentration [16]. Several species of HDL have been identified. These include: 1. lipid poor apo A-I protein chains containing cholesterol and phospholipid. 2. lipid rich discoid particles containing apo A-I. 3. spherical particles containing only apo A-I, and 4. spherical particles containing apo A-I and apo A-II [19]. Some spherical particles also contain apo E. Although most HDL migrates in the alpha region of the gel upon electrophoresis (alpha-HDL), lipid poor apo A-I chains, and discoid particles migrate in the pre-beta region. These are referred to as pre-beta HDL [16,17]. Those particles containing apo A-I alone are called LpAI, and those containing apo A-I and apo A-II are called LpAI:AII. LpAI:AII particles contain apo A-I (two to four copies per particle) and apo A-II in a ratio of 2:1 [17,18]. LpAI particles appear to promote cholesterol efflux from cells, while LpAI:AII particles do not [16,18]. There are also some minor apolipoproteins such as apo A-IV, apo C-II and apo C-III, some of which appear to be associated with ASCVD [19].

HDL_2 is richer in particles containing apo A-I without apo A-II, whereas HDL_3 is richer is richer in particles containing both apo A-I and apo A-II [20]. Some have suggested that large, less dense HDL_2 are more antiatherogenic, but, the associations are statistically modest, at best, and the importance of such a relationship remains unproven [21,22].

Although most HDL is alpha-HDL, about 25 years ago, investigators identified a minor pre-beta migrating HDL [23,24]. This fraction consisted of low molecular weight lipoproteins that contained apo A-I and was more rapidly turning over than alpha-HDL. It was also discovered that this rapidly turning over HDL was discoid in shape (Fig. 1, Table 2) while alpha-HDL are spherical particles (Fig. 1, Table 2). Although it was hypothesized that this pre-beta fraction was of special importance in HDL metabolism, it was about 10 years later that the metabolism of this fraction was put in better perspective. It became clear that the ATP-binding cassette transporter (ABC) A1 channels cholesterol from the liver and other cells to apo A-I [25,26].

A scavenger receptor (SR-B1) was identified that binds spherical HDL with high affinity and mediates cholesterol uptake [28]. Although this receptor can mediate bidirectional flux, the significance of efflux remains unclear [29]. SR-B1 can remove cholesterol by a two–step process where high affinity lipoprotein binding is followed by a transfer of lipid from HDL through the receptor to the cell membrane, after