thematic review

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What have we learned about HDL metabolism from kinetics studies in humans?

Shirya Rashid,* Bruce W. Patterson,† and Gary F. Lewis[1,§]

Department of Cardiology,* McGill University, Montreal, Canada; Department of Medicine,† Division of Geriatrics and Nutritional Science, Washington University School of Medicine, St. Louis, MO; and Departments of Medicine and Physiology,§ University of Toronto, Toronto, Canada

Abstract Plasma measurements of lipids, lipoproteins, and apolipoproteins provide information on the static levels of these fractions without providing key information on the dynamic fluxes of lipoproteins in the circulation. Kinetics studies, in contrast, provide additional information on the production and clearance rates of lipoproteins and the flow of lipids and apolipoproteins through lipoprotein fractions. This information is crucial in accurately delineating the metabolism of HDL in plasma, because plasma concentrations of HDL are the net result of the de novo production and catabolism of HDL as well as the recycling of HDL particles and the contribution to HDL from components of other lipoproteins. Studies aimed at measuring the metabolism of HDL particles have shown that HDL metabolism in vivo is complex and consists of multiple components. Kinetics studies provide a window into the metabolism of HDL, allowing us to better understand the mechanisms of HDL decrease in human conditions and the functionality of HDL particles. Here, we review the progress in our understanding of HDL metabolism derived from in vivo kinetics studies, focusing primarily on studies in humans but also reviewing key studies in animal models.—Rashid, S., B. W. Patterson, and G. F. Lewis. **What have we learned about HDL metabolism from kinetics studies in humans? *J. Lipid Res.* 2006.** 47: **1631–1642.**

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IN VIVO METHODS OF DETERMINING HDL METABOLISM

Individuals with low plasma concentrations of HDL have a significantly increased risk of developing atherosclerotic cardiovascular disease. HDL is thought to protect against atherosclerotic cardiovascular disease in part by reverse cholesterol transport (RCT) from atherosclerotic lesion macrophages to the liver, with the eventual excretion of cholesterol into bile and elimination in feces. The major clinical scenarios in which humans are found to have low HDL include insulin resistance, the metabolic syndrome, hypertriglyceridemic states, and type 2 diabetes. Less common monogenic conditions in which HDL levels are low include such examples as familial hypoalphalipoproteinemia, Tangier disease, mutations of apolipoprotein A-I, and LCAT deficiency.

Investigation of the kinetics of HDL, as opposed to other lipoproteins (VLDL and LDL in particular), presents several difficulties and limitations. Compared with apolipoprotein B (apoB) in VLDL and LDL, most HDL apolipoproteins are readily exchanged between plasma lipoproteins, including between HDL subspecies, exist in equilibrium with free apolipoproteins in the aqueous milieu, may enter cells and organs, and may be cleared separately from the HDL particle (1–3). Although there is certainly considerable exchange of HDL-associated apolipoproteins, kinetics studies take advantage of the fact that at least a certain pool of apolipoproteins remain associated with a single HDL particle or HDL as a class (or subclass) for the duration of the kinetics study. In contrast, the lipid components of HDL, including HDL cholesteryl ester, phospholipids, and triglycerides, undergo protein-mediated interchange between lipoproteins and tissues. In essence, it is easier to track the apolipoprotein components than the lipid components of HDL because of the lesser extent of apolipoprotein versus lipid exchange.

When discussing the principles of tracer methodology, the term "tracee" is defined as the substance of interest, such as apoA-I, to be traced kinetically (4–6). The term "tracer" is a labeled substance, for instance, radioiodinated apoA-I, injected into the body (the "system") to determine the kinetics of the tracee. An ideal tracer has the following characteristics: *1*) its physical and chemical properties are identical to those of the tracee so that it is metaboli-

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[1] To whom correspondence should be addressed.
e-mail: gary.lewis@uhn.on.ca

cally indistinguishable from the tracee; *2*) it does not perturb the system under study; and *3*) it is physically distinguishable by the investigator. Two commonly used approaches for the study of HDL metabolism in humans have been either exogenous labeling of HDL, most commonly performed with a radioisotope (e.g., ^{125}I- or ^{131}I-labeled apolipoproteins), or endogenous labeling of HDL components with radioactive (e.g., [^{3}H]leucine, [^{14}C]cholesterol) or stable (e.g., [$^{2}H_3$]leucine) isotopically labeled precursors.

Exogenous labeling for the assessment of HDL apolipoprotein kinetics

Radioactive iodine tracers have been used either to label all HDL proteins in situ or to specifically label a single purified HDL apolipoprotein of interest, most commonly HDL apoA-I (the major apolipoprotein component of HDL), with subsequent reconstitution with unlabeled HDL (7–10). Because labeling of whole HDL radiolabels all HDL proteins (as does endogenous stable isotope enrichment), the HDL apolipoprotein of interest must be isolated from blood samples taken from the subject after administration of the tracer to measure the specific radioactivity of that single apolipoprotein. The kinetic behavior of radiolabeled pure apolipoproteins reconstituted (exchange-labeled) with HDL may differ from that of apolipoproteins radiolabeled on HDL (whole-labeled), because the former may be more loosely bound to HDL, more easily dissociated from the particle, and rapidly cleared from the circulation. This has indeed been shown to be the case in some (7) but not in other (11) studies.

The radioactive HDL tracer is typically injected into the plasma compartment as a bolus under steady-state conditions (the mass being below that which could perturb the plasma HDL pool size), and production and clearance rates of endogenous HDL are derived from the radioactivity disappearance curve over time by compartmental modeling (11, 12). Radioactive tracer methodology is used infrequently by investigators today to study HDL metabolism, mainly because of ethical concerns regarding radioactivity exposure, although the exposure is extremely low and is on the order of magnitude of many commonly performed diagnostic nuclear medicine scans. Also for this reason, repeated studies in the same subject after interventions (such as dietary or pharmacological interventions) are limited when using radioactive tracers. Another concern is that it is assumed that the isotope labels the tracee in proportion to tracee mass; this is an assumption that must be made to derive conclusions about the tracee, despite some evidence to the contrary (5, 13). Furthermore, the physical and chemical properties of iodinated apoA-I may be altered such that its metabolic properties may potentially differ from those of the native tracee (14). One distinct advantage of using radioactive tracers, however, is that the kinetic behavior of HDL particles whose composition has been manipulated ex vivo can be examined in vivo by exogenous radiolabeling, providing insights into HDL metabolism that cannot be derived from endogenous stable isotope enrichment studies (10).

Endogenous labeling for the assessment of HDL apolipoprotein kinetics

Stable isotopically labeled tracers incorporate stable nuclides that have a low natural abundance into specific positions of tracer molecules, such as [1-^{13}C]leucine, [5,5,5-$^{2}H_2$]leucine, or ring-[$^{13}C_6$]phenylalanine. A high correlation has been reported between stable and radioactive tracer methods for apoA-I kinetics, with a correlation coefficient as high as 0.98 (15, 16). Endogenous labeling with a stable isotopically labeled amino acid has the advantage that the apolipoprotein is biologically labeled from a native amino acid precursor pool such that the physical and chemical properties of the protein are not altered. In fact, labeling of the tracee of interest from amino acid precursors has been the primary method of studying the metabolism of lipoproteins in humans for more than a decade (4). Stable isotopic enrichments are measured using GC-MS or GC-combustion isotope ratio-mass spectrometry instrumentation. Disadvantages of endogenous labeling with stable isotopes include the fact that direct input into the system under study cannot be determined and must be assumed from indirect measures (see below) (4). Furthermore, for particles produced in multiple pathways, such as apoA-I, which is synthesized and secreted by both the intestine and the liver, delineating which particular pathway the particle is labeled from is difficult (4) (this applies to exogenous labeling as well).

Design of stable isotope enrichment studies in humans

Factors to be considered in the design of stable isotope studies include the choice of tracer, infusion, and sampling protocols. In terms of the tracer choice, tracer amino acids used to analyze apolipoprotein synthesis must be abundant in the product protein and should be multiply labeled to increase the sensitivity of mass spectrometry (17). [$^{2}H_3$]leucine is commonly used, and there is a reliable relationship between the isotopic enrichment of plasma leucine and the enrichment of hepatic precursor pools used for apolipoprotein synthesis (18).

Clinical protocols for stable isotopically labeled tracer administration include both bolus injection and primed constant infusion approaches (4). Historically, a constant infusion has been used because higher isotopic enrichments in the desired protein are achieved, making it easier to measure isotopic enrichment above natural abundance background, and because the kinetics analysis using a constant infusion appears simpler compared with a bolus of tracer. A constant infusion approach is suitable for rapidly turning over proteins that do not exchange with the nonplasma space, such as VLDL apoB, because a plateau enrichment in the protein can be achieved in a convenient time frame for a clinical protocol (10–15 h). However, a constant infusion is less practical for slower turning over proteins that do exchange with the nonplasma space, such

as HDL apoA-I or apoA-II, because an isotopic plateau enrichment cannot be achieved in a practical time period. When an isotopic plateau cannot be achieved, it is necessary to make assumptions regarding the isotopic enrichment of the amino acid precursor pool, thereby reducing the reliability of kinetic turnover parameters. Tracer recycling becomes problematic when longer tracer infusion periods are used, although to a certain extent this may be accommodated by appropriate mathematical modeling of the metabolic kinetics. Finally, the duration of sampling that is necessary for a long-term constant infusion is less convenient for both the subject and the staff performing a clinical study.

In contrast, a bolus tracer injection is a more convenient protocol for subjects and clinical staff. A bolus tracer injection provides a richer, dynamic set of kinetics information compared with a constant infusion approach, resulting from increasing and decreasing enrichments in the target protein. A bolus tracer is thus more suitable to study the metabolism of lipoproteins with a relatively slow turnover or that exhibit kinetic heterogeneity resulting from exchange with nonplasma spaces or lipoprotein particle conversion processes. Therefore, a bolus tracer administration is preferred for studies of HDL apoA-I or apoA-II metabolic kinetics (4).

Measuring HDL-mediated RCT

The clinical protocols described above refer to the measurement of HDL protein, typically either apoA-I or apoA-II but also occasionally other HDL-associated apolipoproteins such as apoE and apoC. In contrast, historically there has been great difficulty in measuring the flow of HDL lipid, particularly cholesteryl ester, because of the high degree of exchange of cholesteryl ester between HDL and other lipoproteins via cholesteryl ester transfer protein (CETP)-mediated exchange in the blood and between HDL and tissues via receptor-mediated cholesterol transfer. Thus, it has not been possible to accurately quantify HDL-mediated RCT, occurring from macrophages to the liver and feces in humans, the major process thought to mediate HDL's antiatherosclerotic effect. Similarly, the major tissue source of the HDL cholesterol pool has not been determined. Despite this limitation, many attempts have been made to measure RCT in humans (19). Newer methods include the use of the stable isotope [^{13}C]acetate to measure cholesterol flux between HDL and apoB lipoproteins (20).

Another limitation of HDL cholesteryl ester kinetics studies in the past has been that the majority of studies have been carried out in nonhuman animal models, which have major differences in cholesterol metabolism compared with humans. An example is the mouse, which lacks CETP, an important step in HDL-mediated RCT in humans, in which HDL cholesteryl esters are transferred from HDL to apoB-containing lipoproteins for transport to the liver. Nonetheless, advances have been made in our understanding of RCT from animal studies. One method of measuring RCT in animals has been the administration of [^{3}H]water to measure the rate of peripheral cholesterol synthesis and estimate the peripheral cholesterol efflux at steady state as well as the extent of bile and fecal sterol excretion. This measurement technique has been used in genetically altered mouse models in which single steps of the RCT pathway have been altered (e.g., ABCA1, LCAT, and CETP) (21–24). No effect on the RCT flux, however, was seen in these studies, with the exception of one in which reconstituted HDL particles (with apoA-I and phospholipids) increased RCT to tissues (23). Another more recent method of measuring RCT specifically from macrophages to feces has been developed and used in the mouse model. This method involves the intraperitoneal injection of macrophages labeled with [^{3}H]cholesterol and then measuring the tracer in plasma, liver, bile, and feces. Using this method, it has been shown that mice overexpressing apoA-I and scavenger receptor class B type I have significantly increased macrophage RCT compared with mice deficient in apoA-I (25–27).

Dietschy's group (24) measured the centripetal flow of cholesterol from extrahepatic tissues to the liver in normal mice and transgenic mice expressing simian CETP and determined whether this flow was dependent on plasma HDL cholesterol (HDL-C) concentrations. Sterol synthesis in each organ was calculated as the amount of [^{3}H]water incorporated into digitonin-precipitable sterols per organ (24). Furthermore, the rate of centripetal cholesterol flux was calculated as the sum of the rates of cholesterol synthesis and LDL cholesterol (LDL-C) uptake in extrahepatic tissues (24). In these early studies, the results showed that despite a 4-fold variation in HDL-C concentrations between control mice and those mice maximally expressing CETP, the rate of centripetal cholesterol flow was constant (24). Another set of studies by this group also determined that in mice, centripetal cholesterol flux is independent of HDL (28). That is, centripetal cholesterol flux was similar in control mice and those lacking apoA-I (28).

A limited number of HDL cholesteryl ester studies have been performed in humans (20, 29, 30). In one study (29), for example, [^{13}C]acetate enrichment was measured (after an 8 h intravenous infusion) in cholesterol and cholesteryl ester in various lipoproteins, including HDL, using GC-combustion isotope ratio-mass spectrometry. In that study in fasting normolipidemic subjects, it was found that the major fraction of cholesteryl ester enters plasma via HDL (95%) and disappears through VLDL and LDL catabolism (82%). More recently, Schwartz ,VandenBroek, and Cooper (30) quantified the major in vivo cholesteryl ester transport pathways in humans. Subjects received radiolabeled free cholesterol in HDL, LDL, or particulate (albumin) form and another radioisotope of free or esterified cholesterol or mevalonic acid. These kinetics studies revealed net transport of free cholesterol from HDL to the liver, demonstrating RCT. Furthermore, there was net transport of cholesteryl ester from HDL to intermediate density lipoprotein and VLDL and irreversible cholesteryl ester output from VLDL, intermediate density lipoprotein, and LDL.

HDL APOA-I AND APOA-II KINETICS STUDIES IN HUMANS

The majority of studies in humans have suggested that the rate of clearance of HDL, and not the rate of HDL production, is the main determinant of the variation of HDL-C levels (31–36). More specifically, there is a direct correlation between plasma levels of HDL-C and apoA-I, which shows a strong inverse correlation with the apoA-I fractional catabolic rate (FCR) (10, 31, 32, 35). Exceptions to the predominance of HDL clearance in determining HDL-C concentrations include dietary studies, which have shown that varying the fat content of metabolic diets affects mainly HDL production or transport rates (37). For example, in a study of 13 subjects administered different metabolic diets, a 29% decrease in plasma HDL-C levels observed upon switching from a high to a low intake of saturated fat and cholesterol was significantly correlated with a decrease in apoA-I transport rate but not with changes in apoA-I FCR (37). Similarly, alcohol (38) and estrogen (39) do so mainly by increasing HDL production rates.

Ji et al. (40) showed that although HDL apoA-I and apoA-II FCR are increased in the metabolic syndrome, the plasma concentration of lipoprotein A-I (LpA-I) and LpA-I:A-II during steady state are primarily determined by their production rates. It is possible that in a highly homogeneous population of humans (within a narrow range of body weight, body mass index, insulin sensitivity, and insulin levels), as in this study, the rate of HDL production becomes the predominant factor in determining HDL concentrations in plasma; however, given a broader range of individuals in a population, clearance is the main factor affecting plasma HDL concentrations.

HDL kinetics studies in humans with common low HDL-C disorders

Obesity and insulin resistance. The dyslipidemia that is characteristic of individuals with abdominal obesity and insulin resistance includes an increase in VLDL triglycerides and apoB, accumulation of small dense LDLs, and low plasma levels of HDL-C (41–43). Although no reliable statistics are currently available to indicate precisely what percentage of individuals who have low plasma HDL-C levels also have abdominal obesity, insulin resistance, or hypertriglyceridemia or meet present diagnostic criteria for the metabolic syndrome or type 2 diabetes, when identifying individuals purely on the basis of having low plasma HDL-C one also identifies a population enriched in these other metabolic disorders (44), suggesting that low HDL-C is most commonly linked with these disorders in the general population.

In as much as the plasma HDL-C concentration is closely related to the production and catabolism of apoA-I, both radiotracer and stable isotope kinetics studies in humans have tried to determine the cause of low HDL-C in obesity. In one report, a meta-analysis was performed on 13 published stable isotope studies to compare the kinetics of HDL apoA-I in normolipidemic, lean individuals versus overweight/obese subjects with low HDL-C (45). Compared with lean individuals, overweight/obese subjects had significantly higher HDL apoA-I FCR and production rate, with a greater percentage increase for apoA-I FCR. Furthermore, the apoA-I FCR and production rate were significantly associated with apoA-I concentration. Nonetheless, some caveats must be considered in the report: the analysis did not include equal numbers of male and female subjects, subjects with different levels of insulin sensitivities were grouped together, studies using monocompartmental or multicompartmental modeling were included, and studies carried out under fasting and postprandial conditions were grouped together (45).

A more controlled stable isotope kinetics study of apoA-I was performed in five obese, insulin-resistant women with normal fasting triglycerides without impaired glucose tolerance and in five age-matched control women in the postprandial state (46). In these obese individuals, apoA-I catabolism was increased markedly by 50% versus normal controls, resulting in a significant reduction in plasma apoA-I residence time. Although there was a trend toward an increase in apoA-I production rate in obese, insulin-resistant individuals versus controls, this was not statistically significant. Furthermore, plasma HDL-C levels were significantly correlated with the apoA-I FCR and not with the apoA-I production rate. Thus, the authors concluded that accelerated apoA-I catabolism, and not production, is the main factor responsible for the decrease in HDL-C levels observed in obese, insulin-resistant subjects (46).

Attempts have been made to identify the body fat distribution associated with reduced HDL-C and increased apoA-I FCR in obese individuals. The association of low HDL-C with obesity in fact seems to be strongest with abdominal or central obesity that is characterized by intra-abdominal or visceral fat (47). Similarly, central adiposity, as measured by chest skinfold thickness (48) or truncal girth (34), but not peripheral adiposity, as measured by thigh skinfold thickness (48), has been shown to be positively correlated with apoA-I FCR. To further assess the sublocation of the central obesity associated with apoA-I FCR, the turnover of the two major subfractions of HDL, LpA-I and LpA-I:A-II, was studied under controlled conditions in postmenopausal women (49). Both subcutaneous and intra-abdominal subregions of central fat in fact correlated with the FCR of LpA-I, although not with LpA-I production rate (49).

The impact of interventions aimed at reversing either an overweight profile or the sedentary habits that may have initially led to obesity have shown mixed results. First, weight reduction in overweight, hypertriglyceridemic men achieving body weights within the desirable range (an average loss of 10.6 ± 2.1 kg) resulted in significant reductions in plasma triglyceride levels but only slightly increased HDL-C and apoA-I levels, which were still below normal (50). Similarly, their apoA-I FCRs remained increased. The reason for the poor responsiveness of HDL concentrations to weight reduction (in contrast with the rapid improvements in blood glucose and triglyceride levels seen with even modest weight loss

in obese, insulin-resistant individuals) is not known, but presumably body weight must be normalized or nearly normalized, not merely reduced, to reverse the underlying factors responsible for a decrease of HDL plasma concentrations in obese individuals. In contrast, the effect over a 1 year period of exercise training without weight loss on HDL metabolism was examined in overweight men (51). The men consumed defined diets in a metabolic kitchen and performed supervised endurance exercise during the study period. Significant increases in HDL-C and apoA-I and decreases in triglycerides and apoB (10%, 9%, −7%, and −10%, respectively) were observed. In addition, the FCR of HDL-associated apoA-I decreased significantly by 7%, whereas the apoA-I synthetic rate increased by 13%. Overall, the metabolic changes observed in the context of prolonged exercise training in overweight men were modest.

Impaired fasting glucose, impaired glucose tolerance, metabolic syndrome, and type 2 diabetes mellitus. Impaired glucose tolerance (IGT) is characterized by insulin resistance and hyperinsulinemia, and individuals with IGT have a very high risk of converting to type 2 diabetes mellitus (T2DM). In patients with IGT and those with T2DM, HDL-C levels are generally 15–30% lower than in normal individuals (52, 53). To examine whether patients in the transitional IGT state without frank hypertriglyceridemia and before frank T2DM have altered HDL metabolism, the in vivo kinetics of HDL apoA-I and apoA-II metabolism were studied using the stable isotope L-[$^{13}C_6$]phenylalanine (52). In IGT subjects compared with normal controls, the HDL apoA-I FCR was increased substantially, whereas the apoA-I production rate, apoA-II FCR, and apoA-II production rate were not different. Furthermore, plasma HDL-C and apoA-I levels were significantly lower in IGT subjects compared with controls and were negatively correlated with the apoA-I FCR, whereas triglyceride levels were higher. Overall, HDL metabolic abnormalities constitute early defects before the development of frank T2DM.

Stable isotopically labeled tracer studies in patients with established T2DM not treated with exogenous insulin have also shown significantly increased HDL apoA-I FCR, whereas the HDL apoA-I production rate was not different from that in controls (54). A study in T2DM patients in whom the kinetics of apoA-I and apoA-II in the postprandial state were compared (using stable isotope tracers) with that in control subjects before and 2 months after the introduction of insulin therapy resulted in significant improvements in glycemic control but no change in insulin sensitivity or HDL apoA-I metabolism (55). Neither plasma HDL-C nor apoA-I levels improved, and an increase in HDL apoA-I FCR above that of normal controls was not significantly decreased after insulin therapy. Previous reports in T2DM patients on insulin therapy similarly noted either no change or moderate alterations in HDL-C and apoA-I levels with insulin therapy (56–58).

Mechanisms by which HDL kinetics are altered in insulin-resistant states. The kinetics studies described above in humans have been extremely instructive in highlighting the fact that increased clearance of HDL-associated apoA-I is the major abnormality of HDL metabolism in these highly prevalent metabolic conditions and likely accounts for the majority of HDL-C decrease. In the search for the mechanisms leading to enhanced HDL apoA-I FCR in insulin-resistant subjects, Pietzsch et al. (52) examined factors intrinsic to HDL particles (HDL composition) and extrinsic to HDL particles (CETP and HL activities). HDL particles of subjects with IGT were significantly enriched in triglycerides and phospholipids and depleted of cholesteryl ester and protein. HDL triglyceride mass correlated negatively with HDL cholesteryl ester and apoA-I mass. Similarly, in obese, insulin-resistant individuals with normal glucose tolerance and in patients with T2DM, increases in the HDL triglyceride-to-cholesterol ratio have been observed consistently (46, 54).

In all three insulin-resistant states (obesity, IGT, and T2DM), the mechanisms of the reduced HDL-C and apoA-I and enhanced HDL apoA-I FCR are thought to share common pathways. CETP-mediated neutral lipid exchange between the expanded pool of triglyceride-rich lipoproteins in these conditions results in the enrichment of HDL particles with triglycerides and the depletion of cholesteryl ester mass (59, 60). A number of studies have shown strong positive correlations between HDL triglyceride content (or the triglyceride-to-cholesterol ratio in HDL) and apoA-I FCR (46, 54, 55). Such correlations, however, do not prove a cause-and-effect relationship between HDL triglyceride content and apoA-I FCR.

More direct evidence was required to prove that HDL compositional change, of which triglyceride enrichment is one prominent feature, plays a role in modulating HDL metabolism. For example, in vitro incubation of HDL with CETP and VLDL to triglyceride-enrich the HDL particles resulted in the dissociation of apoA-I from HDL (61). Similarly, apoA-I in HDL particles that are enriched in triglycerides have been shown to be catabolized more rapidly than apoA-I in native HDL in humans and animal models (10, 62–64). The triglyceride-enriched particles are more susceptible to the lipolytic activity of HL, located at the liver sinusoids (19). HL lipolyzes the HDL particles, resulting in apoA-I being more loosely associated with the particles and cleared more readily by the kidney (7) (**Fig. 1**). It should be noted, however, that the enhanced CETP-mediated deposition of triglycerides onto HDL that occurs in insulin-resistant states produces other compositional changes in HDL in addition to triglyceride enrichment of the particles. The process reduces cholesteryl ester and free cholesterol in HDL (62) and alters HDL size, charge, and phospholipid and apolipoprotein composition (particularly apoE and apoC content) of the HDL particle (10). Thus, triglyceride enrichment may simply be a marker of a compositionally altered HDL particle that occurs in the setting of hypertriglyceridemia and enhanced CETP-mediated lipid exchange. The molecular mechanisms of increased HDL apoA-I FCR and HDL-C and apoA-I decreases in these conditions are not well understood.

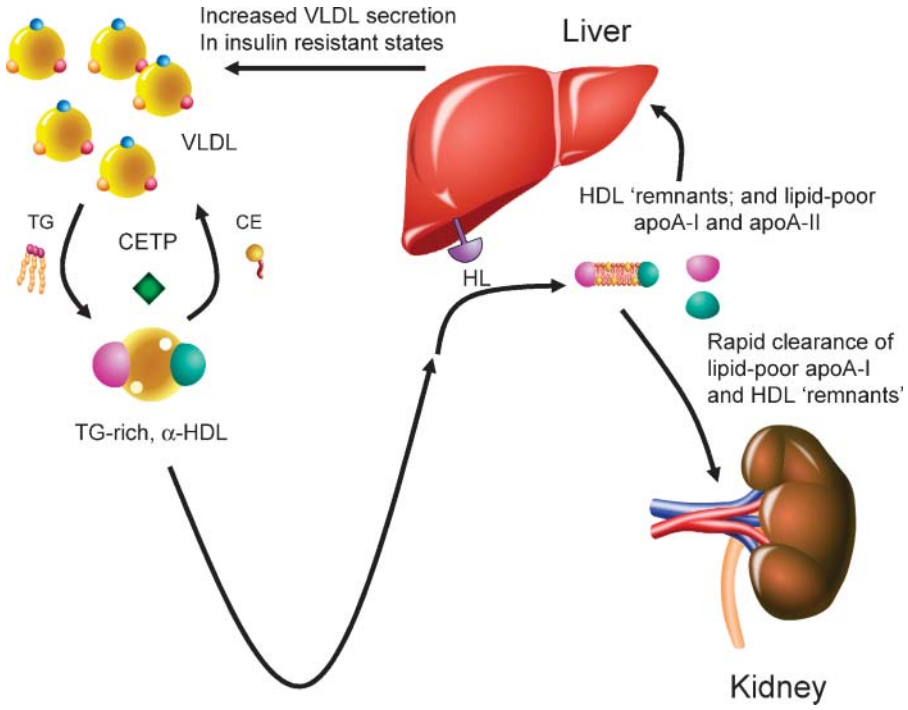

Fig. 1. Mechanism of decreasing HDL cholesterol (HDL-C) and HDL apolipoprotein A-I (apoA-I) in insulin-resistant and other hypertriglyceridemic states. Insulin-resistant states are associated with an increase in VLDL production and postprandial chylomicronemia. Cholesteryl ester transfer protein (CETP)-mediated heteroexchange of HDL cholesteryl ester (CE) with triglyceride (TG) in the expanded pool of these TG-rich lipoproteins results in CE depletion and TG enrichment of HDL particles. HL, which is also increased in insulin-resistant states, modifies TG-rich HDL, releasing lipid-poor apoA-I and forming HDL "remnant" particles. Lipid-poor apoA-I can either be recycled to form mature spherical HDL particles or filtered by the renal glomerulus and then degraded by proximal renal tubular cells. HDL remnants (α-migrating, lipolytically modified HDLs) may also bind to putative receptors in liver or kidney that mediate HDL holoparticle uptake, internalization, and degradation. The combination of TG enrichment of HDL and increased hepatic lipase activity plays an important but not exclusive role in the decrease of plasma HDL-C concentration in insulin-resistant states.

HDL kinetics studies in rare human disorders characterized by low HDL-C and apoA-I (hypoalphalipoproteinemia)

The in vivo metabolism of HDL particles has been examined in humans who have rare HDL genetic variants associated with low plasma HDL concentrations. Understanding the kinetic behavior of these patients' HDL particles has contributed to the understanding of how these disorders affect HDL metabolism and has furthered our understanding of the process of RCT. Monogenic disorders causing low HDL-C include variants in the genes for the cholesterol and phospholipid transporter ABCA1, the major HDL apolipoprotein apoA-I, and LCAT, the enzyme responsible for the esterification of free cholesterol in the HDL particle after removal from the plasma membrane of donor tissues. These proteins play a critical role in early steps of RCT and have potentially major effects on the development of atherosclerosis. To begin with, *ABCA1* encodes a protein involved in the transport or efflux of cholesterol and phospholipids from cells to HDL. A defect in one *ABCA1* allele causes the disorder familial hypoalphalipoproteinemia (FHA), which is characterized by moderately low HDL-C and apoA-I and premature coronary heart disease (65). To examine whether the decline in HDL levels in FHA is attributable to impaired HDL production or increased HDL catabolism, the kinetics of plasma apoA-I and apoA-II were examined in two related FHA patients and four control subjects using a primed constant infusion of deuterated leucine (66). Production rates of apoA-II were normal in both patients and controls, whereas the residence times of mature apoA-I and apoA-II were significantly less in FHA patients. Thus, the enhanced catabolism of immature apoA-I and apoA-II can explain the marked decline of HDL-C and apoA-I levels observed in FHA patients. Note that this is one of a number of examples in which the defective formation of HDL particles that leads to abnormal HDL composition ultimately affects their rate of removal from the circulation. In contrast, the production and catabolic rates of proapoA-I (the precursor protein of apoA-I) were normal in FHA patients and associated with normal proapoA-I levels (66).

Individuals who have a defect in both alleles of ABCA1 are diagnosed as having Tangier disease. Patients with Tangier disease have extremely low levels of plasma apoA-I and HDL-C and are characterized by a marked decline in

the efflux of lipids onto HDL particles (67). Whereas the major apolipoprotein from HDL in normal individuals is mature apoA-I, in this condition there is a relative increase of the precursor proapoA-I (which has six additional amino acids at the N terminus that must be cleaved to form mature apoA-I) versus mature apoA-I (68). To determine the mechanism of the decline in apoA-I and the relative increase in proapoA-I in Tangier disease, kinetics studies were conducted in normal and Tangier disease subjects using autologous apoA-I isoproteins (68). This revealed a significantly faster rate of catabolism of both proapoA-I and mature apoA-I in individuals with Tangier disease versus unaffected individuals. Furthermore, the relative increase in proapoA-I in the patients was not attributable to a defective conversion of proapoA-I to mature apoA-I, as Tangier disease subjects had a normal fractional rate of conversion of proapoA-I to mature apoA-I, but was the result of a marked decrease in mature apoA-I caused by its enhanced catabolism.

ApoA-I_{Milano} is a genetic variant of apoA-I in which a cysteine is substituted for arginine at amino acid 173 (69). Subjects heterozygous for apoA-I_{Milano} represent an interesting phenotype in that despite having low levels of plasma HDL-C and apoA-I, they do not have the anticipated increase in vascular risk (65). Kinetics studies with radiolabels have been performed in these subjects to delineate the cause of low HDL in apoA-I_{Milano}. In a study by Roma et al. (69), both normal and mutant apoA-I were radiolabeled and simultaneously injected into two patients and two normal subjects. In the normal subjects, apoA-I_{Milano} was cleared more rapidly than normal apoA-I, demonstrating that apoA-I_{Milano} is kinetically abnormal and that it has a shortened residence time in plasma. In contrast, in apoA-I_{Milano} subjects, both forms of apoA-I were catabolized more rapidly than normal (likely attributable to the association of labeled native apoA-I with HDL containing a rapidly catabolized, unlabeled, abnormal apoA-I_{Milano} in these affected subjects), whereas production rates were normal. Synthetic HDL-like particles containing apoA-I_{Milano} are currently under development as antiatherosclerotic therapies.

Other mutations at or near the gene for apoA-I have been identified and associated with hypoalphalipoproteinemia. For example, a mutant protein termed apoA-I_{Iowa} has been isolated in a kindred in which an arginine is substituted for a glycine at residue 26 in apoA-I (70). The in vivo metabolism of apoA-I_{Iowa} was compared with that of normal apoA-I in two heterozygous apoA-I_{Iowa} subjects and two normal controls (70). The two proteins were radioiodinated and simultaneously injected into the subjects. Results of the kinetic die-away clearance curves indicated that normal apoA-I was cleared more rapidly in heterozygous apoA-I_{Iowa} subjects compared with normal controls. As is the case with apoA-I_{Milano} discussed above, this is likely attributable to the association of labeled native apoA-I with HDL containing a rapidly catabolized, unlabeled, abnormal apoA-I_{Iowa} in these affected subjects. Moreover, mutant apoA-I_{Iowa} was cleared approximately twice as fast as normal apoA-I in all study subjects. Nonetheless, the cumulative urinary excretion of apoA-I_{Iowa}-associated radioactivity after 2 weeks was less than that associated with normal apoA-I (44% vs. 78%, respectively). This indicates extravascular sequestration of apoA-I_{Iowa} with less production of by-products of protein breakdown and amino acid catabolism. Such sequestration of apoA-I_{Iowa} is consistent with the formation of apoA-I_{Iowa}-containing amyloid deposits in heterozygous carriers of apoA-I_{Iowa}.

In addition, a polymorphism has been detected for the *Pst*I restriction endonuclease near the apoA-I gene, with a 3.3 kb fragment, occurring in higher frequency in the kindred with FHA (hypoalpha apoA-I) (71). ApoA-I was isolated from three unrelated individuals with hypoalphalipoproteinemia and the 3.3 kb *Pst*I polymorphism of the apoA-I gene and from seven normal controls (70). Normal apoA-I and hypoalpha apoA-I were radioiodinated and injected simultaneously into each subject. Analysis of the kinetics curves demonstrated that both forms of apoA-I were catabolized at the same rate in the same subject; however, there were significant differences in apoA-I kinetics between hypoalphalipoproteinemic subjects and controls. That is, the hypoalphalipoproteinemic subjects had a reduced residence time (an increased FCR) for both normal apoA-I and hypoalpha apoA-I compared with normal controls. The synthetic rate of apoA-I in hypoalphalipoproteinemic subjects, in contrast, was normal. Thus, the reduced plasma levels of apoA-I in these subjects is attributable to enhanced apoA-I catabolism.

At least 30 different mutations in *LCAT* have been reported (72). Classic (complete) LCAT deficiency and fish-eye disease (partial LCAT deficiency) are like apoA-I_{Milano} in that they are genetic syndromes associated with markedly decreased HDL-C but without the associated increased risk of atherosclerotic cardiovascular disease (73). Both forms of LCAT deficiency are associated with reduced cholesterol esterification in plasma (65). The kinetics of apoA-I and apoA-II were investigated in a patient with classic LCAT deficiency and in four patients with fish-eye disease (73). The results showed that the mean plasma residence times of apoA-I and, more so, apoA-II were decreased in the patients versus controls, demonstrating faster apoA-I catabolism in the patients. ApoA-I in LpA-I:A-II, moreover, was markedly faster than apoA-I in LpA-I. Because LpA-I is believed to be more atheroprotective than LpA-I:A-II, the selective hypercatabolism of particles with apoA-II in LCAT-deficient patients may explain why these individuals are not at increased risk for developing premature cardiovascular disease.

HDL kinetics studies in rare human disorders characterized by high HDL-C and apoA-I (hyperalphalipoproteinemia)

In addition to the rare genetic variants causing low plasma concentrations of HDL described above, investigations of two other genetic variants resulting in either normal or above-normal HDL concentrations have furthered our understanding of HDL metabolism. These two genetic variants are HL deficiency and CETP deficiency.

Kinetics analyses were carried out in three complete and three partial HL-deficient subjects (74). The partial HL-deficient subjects were matched with eight normotriglyceridemic controls, and the complete HL-deficient subjects were matched with two hypertriglyceridemic controls (because complete HL deficiency is associated with hypertriglyceridemia). A 12 h primed-constant infusion of deuterated leucine was used to determine the in vivo kinetics of apoA-I and apoA-II in the fasted state. Complete HL deficiency in males (two siblings) was associated with enlarged, triglyceride-rich HDL particles and a 21% lower apoA-I FCR compared with hypertriglyceridemic, gender-matched control subjects. Complete HL deficiency in the female proband was associated with a 47% reduction in apoA-I FCR. Partial HL deficiency was not associated with significant differences in HDL profiles, composition, and kinetics compared with control subjects. These results indicate that HL activity is required for normal HDL catabolism.

Slower catabolism of apoA-I has also been observed in individuals homozygous for CETP deficiency. In general, individuals with CETP deficiency have increased plasma levels of HDL-C and apoA-I (75). The in vivo metabolism of apoA-I was investigated in three individuals with CETP deficiency (two unrelated heterozygotes and one homozygote) and four control subjects using both exogenous (radioiodination) and endogenous (primed constant infusion of [$^{13}C_6$]phenylalanine for 16 h) labeling techniques. Although the kinetics of apoA-I in the CETP-deficient heterozygotes were not different from those in control subjects, the turnover of apoA-I was markedly slower in both HDL_2 and HDL_3 in the CETP-deficient homozygotes, with reduced FCR compared with controls.

HDL kinetics in humans administered pharmacotherapies

The effects of several pharmacotherapies on HDL metabolism have been investigated. For example, 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, or "statins," used primarily to decrease LDL-C levels, have been found in some in vitro studies to increase apoA-I synthesis and to have minor inhibitory effects on CETP and hepatic lipase, factors that could affect HDL metabolism (76, 77). Clinically, statins generally increase plasma HDL-C concentrations by between 5% and 10%, with minor differences in HDL-increasing effects reported between various statins. Most studies in humans have failed to demonstrate a consistent alteration of either production rate or catabolic rate of apoA-I with statins (78–81), but one study demonstrated an increase in apoA-I production with pravastatin treatment (39).

Pioglitazone, a member of the thiazolidinedione class of peroxisome proliferator-activated receptor γ agonists, which are insulin-sensitizing agents indicated for the treatment of T2DM, increases HDL-C by up to 15% (82), although the mechanism of its HDL-increasing effect is currently not known. In one human study that examined the metabolism of HDL apoA-I using stable isotope enrichment methodology in eight T2DM individuals treated with pioglitazone for 14–16 weeks, there was no significant change in HDL apoA-I kinetic parameters, despite a significant increase of plasma HDL-C concentration by 14% (83). Rosiglitazone, another member of the thiazolidinedione class of antidiabetic agents, has been shown in animal studies to inhibit the transcription of hepatic lipase (84), raising the possibility that a reduction in hepatic lipase enzymatic activity may be one mechanism whereby this class of therapeutic agents increases plasma HDL levels.

Fibrates, which are peroxisome proliferator-activated receptor α agonists, are indicated for the treatment of hypertriglyceridemia and low HDL-C. They have been shown to induce the expression of genes encoding apoA-I, apoA-II, and ABCA1, thereby potentially altering the synthesis of HDL-containing apoA-I and both apoA-I and apoA-II (78). Indeed, the fibrate fenofibrate significantly increased plasma levels of HDL_2 and HDL_3 cholesterol, apoA-I, and apoA-II in dyslipidemic men with the metabolic syndrome (78). The pool size of apoA-I was increased by fenofibrate by inducing a greater increase in apoA-I production rate than its effect on increasing apoA-I FCR. The stimulatory effect of fenofibrate and gemfibrozil on apoA-I production has been confirmed by others (79, 85, 86), whereas bezafibrate did not significantly alter apoA-I or apoA-II turnover (87). One study (86) demonstrated an increase in FCR with fenofibrate therapy, although the increase in FCR was much less than the increase in production rate.

More recently, pharmacological inhibition of CETP by torcetrapib was found to increase the steady-state levels of HDL-C by as much as 74% in normolipidemic individuals and by up to 53% in patients with low HDL-C (88). The kinetics of HDL metabolism with torcetrapib were investigated in three groups of subjects administered stable isotopes: those taking 20 mg of atorvastatin for hypercholesterolemia plus torcetrapib; normocholesterolemic subjects receiving 120 mg of torcetrapib once daily (QD) alone without atorvastatin; and individuals with normal LDL-C levels receiving 120 mg of torcetrapib twice daily (BID) alone without atorvastatin (89). Compared with placebo, HDL apoA-I increased by 136% in the atorvastatin plus torcetrapid cohort, by 153% in the QD torcetrapib cohort, and by 382% in the BID torcetrapib group (89). All increases were found to be in the largest α1-migrating HDL. CETP inhibition, moreover, did not alter HDL apoA-I production rates but did reduce HDL apoA-I FCR significantly in the atorvastatin, QD, and BID groups (by 7%, 8%, and 21%, respectively). It is believed that an increase in HDL particle size and density (via an increase in HDL cholesteryl ester content) contributed to the increased stability of HDL particles by torcetrapib (34, 89). The question was next posed whether by reducing apoA-I FCR, torcetrapib reduces the rate of RCT, as assessed indirectly via fecal concentrations of neutral sterols and bile acids (89). Neither fecal sterol nor bile acid concentration was altered by torcetrapib treatment using this indirect method of assessing RCT, suggesting that RCT was not altered.

KINETICS STUDIES OF HDL SUBFRACTIONS

HDL particles are heterogeneous in terms of their lipid and apolipoprotein composition, size, and density. They can be divided into particles containing solely apoA-I (LpA-I) and particles containing both apoA-I and apoA-II (LpA-I:A-II) (90). It is important to bear in mind that apoA-I and apoA-II are exchanged between lipoprotein subfractions, making it difficult to analyze their metabolism separately from one another. Nevertheless, studies have investigated whether apoA-I in both types of particles and apoA-II in LpA-I:A-II have different metabolic fates. The results indicate that they do (73, 91). In general, several studies have shown that levels of apoA-I are determined primarily by its rate of catabolism (11, 32, 33), whereas apoA-II levels are determined by its rate of production. More specifically, Ikewaki et al. (36) examined the effect of apoA-I and apoA-II kinetics on the distribution of LpA-I and LpA-I:A-II particles in normolipidemic individuals (excluding the bottom and top percentiles for HDL-C levels). They found that the levels of apoA-I in LpA-I particles were strongly correlated with the rate of apoA-I catabolism. Furthermore, levels of apoA-I in LpA-I:A-II particles were most strongly correlated with the rate of apoA-II production in multivariate analysis. This can be interpreted to indicate that increased apoA-II production results in a shift in apoA-I from LpA-I to LpA-I:A-II particles, thereby decreasing the levels of LpA-I particles and increasing the levels of LpA-I:A-II particles. Thus, the rate of apoA-II has a strong effect on the distribution of apoA-I between LpA-I and LpA-II particles and possibly affects the antiatherosclerotic effect mediated by HDL particles. Interestingly, a recent study that used a new model to study HDL particle kinetics in humans showed, in addition to the anticipated accelerated catabolism of HDL apoA-I- and apoA-II-containing particles in men with the metabolic syndrome, an increase in the production rate of HDL particles containing exclusively apoA-I (LpA-I) (40).

The heterogeneity of LpA-I and LpA-I:A-II particles was also demonstrated in a series of radioisotope experiments in normolipidemic subjects (91). The investigators showed that the turnover of apoA-I in LpA-I was much faster than the turnover of apoA-I in LpA-I:A-II. These investigators also showed the metabolic heterogeneity of HDL_2 versus HDL_3 particles isolated by ultracentrifugation. ApoA-I in HDL_3 was found to be catabolized at a faster rate than apoA-I in HDL_2. There was also an increase in radioactivity solely in the HDL_2 subfraction in the first few hours of the kinetics experiments, indicating a net conversion of HDL_3 to HDL_2.

HDL particles have also been separated into preβ1 and α subclasses using fast-protein liquid chromatography after endogenous labeling with stable isotopes (92). Preβ1 particles are the initial acceptors of cholesterol and typically constitute 5–7% of total apoA-I in humans. These particles are synthesized by the liver and intestine and are converted to spherical HDL_3 particles by LCAT. Using the methodology described above, the kinetics of preβ1 and α HDL particles were compared in six T2DM individuals and six normal controls (93). The results showed significantly faster apoA-I FCRs in diabetic individuals in both preβ1 and α HDL. In addition, the synthetic rate of preβ1 HDL was increased slightly and there was a greater rate of recycling of α to preβ1.

CLINICAL SIGNIFICANCE

As a result of the cardioprotective effects of HDL, it is imperative that the mechanisms of HDL metabolism be elucidated. Indeed, both exogenous and endogenous labeling HDL kinetics studies in humans have allowed us to better understand HDL metabolism and the mechanisms of HDL decrease in human conditions, providing a window into HDL metabolism that goes far beyond the measurement of plasma HDL-C or apoA-I concentrations. For example, kinetics studies have shown that increased HDL clearance is the major mechanism underlying the highly prevalent forms of low HDL-C and apoA-I observed clinically (i.e., in the metabolic syndrome, obesity, and T2DM). Given the complexity and expense of the methods used to carry out kinetics studies, they are unlikely to become clinically useful diagnostic tests outside of the research setting for individuals with HDL abnormalities. Although there are obvious advantages of stable isotope enrichment over radiolabeled tracer methods (i.e., avoidance of radioactivity exposure and less modification of the HDL particle), no method is perfect, and there are ongoing limitations of stable isotope methodologies and some unique advantages of radiotracer methods (such as the ability to test the in vivo effects of compositional modifications of HDL particles by exogenous labeling of modified HDL or synthetic HDL particles).

Although kinetics studies of HDL apolipoprotein metabolism have been highly instructive and have provided great insights into HDL metabolism, additional major advances will come if we are able to accurately quantify the flux of cholesterol between tissues and through the plasma lipoprotein system. Knowing, for example, that a particular genetic, pharmacological, or lifestyle intervention enhances macrophage-specific RCT, which has been shown to be critically important in protecting against atherosclerosis (reviewed in 19), will be invaluable in designing and testing new antiatherosclerotic therapies. Fortunately, exciting new methods to examine this aspect of HDL metabolism in animals are beginning to be applied to human research. As our appreciation of the highly prevalent epidemic of abdominal obesity, with its links to inflammation, insulin resistance, low HDL, hypertriglyceridemia, hyperglycemia, and myriad other metabolic abnormalities, begins to occupy center stage in the causation of atherosclerosis and T2DM, HDL kinetics studies will continue to play an important role in research into pathophysiology and the testing of new therapies.

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