History of Discovery

Triglyceride-Rich Lipoproteins and Plasma Lipid Transport

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Abstract—This memoir provides a history of the triglyceride-rich lipoproteins of blood plasma over the last half-century. As precursors of low-density lipoproteins and in their own right, triglyceride-rich lipoproteins are essential to the formation of atherosclerotic plaques and to consequent ischemic vascular disease. The author recounts research at the National Heart Institute during 1953 to 1956 and continuing thereafter at the University of California San Francisco. Emphasis is placed on key insights arising from investigations of human disease, the interplay of fatty acid and triglyceride-transport involving the liver, small intestine, adipose tissue and muscle, and the role of the liver in the synthesis and catabolism of atherogenic lipoproteins. (Arterioscler Thromb Vasc Biol. 2010;30:9-19.)

Key Words: chylomicrons ■ very low-density lipoproteins ■ apolipoproteins ■ lipoprotein receptors ■ atherosclerosis

In 1951, I was one of 8 residents at East Coast medical schools recruited by James Shannon to the National Heart Institute as Clinical Associates to provide care for patients in the Clinical Center of the National Institutes of Health (NIH). At the time, many house officers were being drafted into the U.S. Army for service in Korea. To obviate loss to a “mash” unit, we immediately joined the U.S. Public Health Service. Owing to delays in opening the Clinical Center, I was able to finish my residency at New York Hospital/Cornell Medical Center, arriving in Bethesda in 1953, where by chance I admitted the first inpatient on July 1. In addition to caring for patients our group was expected to engage in research. We were advised to “look around” and select a basic science laboratory to join. During planning for the Clinical Center, Shannon, aware that virtually no NHI scientists were doing “heart” research, asked laboratory heads to suggest appropriate disease-related areas. Christian B. Anfinsen, a protein chemist (and later Nobel laureate for his work on protein folding), perused the literature and suggested two topics related to plasma lipids. The first was to investigate the lipemia “clearing factor.” At the University of Rochester, Paul Hahn had made a serendipitous observation: in dogs given intravenous heparin, the opalescence of blood plasma occurring postprandially rapidly disappeared.1 Anfinsen suspected that a protein might be released into the blood by heparin. The second was to investigate the plasma lipoproteins recently described by John Gofman and his students at the University of California Berkeley. They discovered that lipoproteins can be separated from serum by ultracentrifugation and quantified in an analytic ultracentrifuge; and they had reported that a low-density group of these giant macromolecules are risk markers for coronary heart disease.2 As a result, Anfinsen was asked to organize a new Section in the Laboratory of Metabolism in 1951. Of the 8 who arrived in 1953, Donald Fredrickson, Robert Gordon, and I joined this Section.

Research at the National Heart Institute (1953 to 1956)

I had been made aware of plasma lipoproteins and their possible relationship to atherosclerotic disease as a house officer at Cornell. David Barr, the Chair of Medicine, Howard Eder, a young Assistant Professor, and Ella Russ, a senior technician, were applying salt-precipitation methods developed during World War II by Edwin Cohn at Harvard to separate plasma protein fractions. Two fractions, respectively, were known to contain alpha- and beta-migrating lipoproteins and Barr, Eder, and Russ were measuring their content of cholesterol and phospholipids. They found beta lipoprotein-cholesterol concentration to be elevated in patients with coronary heart disease, whereas that of alpha lipoprotein-cholesterol was reduced.3 One day Barr showed me a highly lactescent plasma sample from a postpartum woman and asked whether I thought the plasma of her newly born infant was also lactescent (of course it was not!). In Bethesda, I elected to work with Joseph Bragdon, a pathologist who had just joined Anfinsen’s new group. He generously offered me space in his laboratory before research facilities in the Clinical Center were available. Bragdon had actually been studying heart disease in another NIH Institute where he was injecting lipoproteins into rabbits in an attempt produce intimal lesions4 and was quantifying lipid components of plasma and blood vessels for this research; also, he had recently acquired a preparative ultracentrifuge. We initiated studies in rats on the effects of the heparin-antagonist, protamine sulfate, on plasma lipoproteins separated by ultracentrifugation (including lipoprotein-triglycerides by a indi-
rect method that Bragdon had developed). I began to characterize and quantify the chemical components of ultracentrifugally separated subfractions of low-density and high-density lipoproteins in Clinical Center outpatients with plasma lipid abnormalities. Eder joined Anfinsen’s section in 1954 and worked with us. We did not anticipate that our method of sequential ultracentrifugation would become so widely used to quantify lipoproteins in clinical research. At the same time, Gordon, who was interested in plasma proteins and was aware that albumin carries unesterified fatty acids (UFA), had initiated his path-breaking research on the lability of UFA levels, and Fredrickson (who had studied cholesterol metabolism with Ivan Frantz at Harvard) was continuing that line of research. I also initiated clinical studies in healthy inpatient volunteers on postprandial changes in lipoproteins and research on chylomicron metabolism in dogs with Fredrickson (who was experienced in radioisotope techniques). We postulated that the clearing factor (which Edward Korn, a biochemist recruited by Anfinsen from the University of Pennsylvania, was showing to have triglyceride-lipase activity) is involved in chylomicron-triglyceride clearance. We found that labeled palmitate in lymph chylomicron-triglycerides injected intravenously is rapidly cleared from the blood and extensively hydrolyzed to UFA. We were particularly excited to show that labeled palmitic acid, bound to albumin, is cleared from dog plasma with a first-order half-time of only 2 minutes. I had been reading the work of Kenneth Zierler at Johns Hopkins, who had shown that human forearm muscle at rest mainly burns lipid (as estimated by gas-exchange data) but had been unable to identify the expected utilization of a plasma lipid component. I suggested to Gordon that UFA could be the “culprit,” which he soon demonstrated in similar work, also showing that UFA are released from adipose tissue.

In 1954 I became aware of a kindred in which 3 of 6 siblings had creamy serum. In the Clinical Center, I demonstrated that their lipemia was highly sensitive to dietary fat and unaffected by sustained administration of heparin. Figure 1. Gordon and I then showed that unlike normal individuals or other patients with idiopathic hyperlipemia, their plasma levels of UFA failed to increase after intravenous injection of heparin. Furthermore, they had a profound defect in clearance of intravenously administered chylomicrons that was unaffected by prior injection of heparin or protamine sulfate. Also, triglyceride hydrolysis activity in postheparin plasma, assayed with coconut oil emulsion, was much reduced. Korn had shown that the clearing factor (which he named “lipoprotein lipase”) requires a “coprotein,” but we found no evidence for lack of coprotein in the affected individuals. The recessive character of the abnormality, evident in the immediate family, led us to propose homozgyosity for lipoprotein lipase deficiency as the first inherited disorder of lipoprotein metabolism. Issues related primarily to substrate-specificity of the enzyme delayed full acceptance of this proposal (postheparin plasma from the affected individuals, although virtually lacking hydrolysis activity against chylomicron-triglycerides in vivo, had some activity against coconut oil emulsion-triglycerides). While a graduate student at Vanderbilt University, Robert Hamilton subsequently showed that a lipase released from liver by heparin readily hydrolyzes triglycerides in coconut oil but has virtually no activity against chylomicron-triglycerides. Full acceptance came during the 1970s with the application of a method to distinguish lipoprotein lipase and hepatic lipase in human postheparin plasma by Ronald Krauss and Fredrickson and the advent of molecular biology.

**UCSF and the Cardiovascular Research Institute. Phase I: Plasma Lipid Metabolism (1956 to 1971)**

In 1956 I moved to the University of California San Francisco as an Assistant Professor of Medicine, where a Cardiovascular Research Institute was to be built in the new Moffit Hospital. I soon received a 5-year award as an Established Investigator of the American Heart Association to continue my research on triglyceride-rich lipoprotein (TRL) and fatty acid metabolism and a research grant from the National Heart Institute. I was given substantial clinical and teaching duties, but the Investigator award was critical to providing time to devote most of my effort to initiating research in temporary space. The next year Julius Comroe from the University of Pennsylvania, an accomplished cardiopulmonary physiologist, became Institute Director, and in 1958 the new enterprise began. In 1960, the first-year basic science departments moved to the San Francisco campus from UC Berkeley. At the same time, the Institute’s founding investigators received a programmatic grant from the National Heart Institute under which Comroe fostered a collaborative atmosphere in an institution with limited traditions in laboratory-based research among its clinical departmental faculty.

When I came to UCSF, it was evident that chylomicron-triglycerides account for the mass transport of fatty acids from gut to body tissues via UFA. Bragdon and Gordon had shown that a large fraction of injected UFA is rapidly taken up by rat liver. From ultracentrifugal studies it was also evident that human hypertriglyceridemia commonly reflects...
increased levels in very low-density lipoproteins (VLDL), not chylomicrons. Although VLDL-triglycerides were only mildly increased in our subjects with lipoprotein lipase-deficiency, their lability\(^10\) suggested the potential to mediate substantial transport of endogenously produced fatty acids. In my initial effort to determine the source of VLDL-triglycerides, I used a low-efficiency end-window counter to measure \(^{14}\text{C}-\text{palmitate}. This tedious procedure was obviated when other faculty and I obtained the first liquid scintillation counter at UCSF (but lacking an automatic sample changer). Experiments in intact and hepatectomized dogs (with Alan Goldfien) and rabbits (with James Felts, who joined me in the CVRI in 1959) showed that VLDL-triglycerides derived from UFA (now called free fatty acids [FFA]), are produced by the liver.\(^{23,24}\) Kinetic studies in normotriglyceridemic and hypertriglyceridemic humans then showed that FFA are the major precursors of triglyceride fatty acids (TGFA) in VLDL.\(^{25}\) In humans and rabbits, VLDL-TGFA were the precursors of TGFA in low-density lipoproteins (LDL) and high-density lipoproteins (HDL).\(^{24,25}\) Triglycerides in rabbit VLDL were transferred to the other lipoproteins during incubation in vitro (shown many years later to be mediated by cholesteryl ester transfer protein [CETP]). In fasted and subsequently refed rabbits expressing high activity of lipoprotein lipase in fat cells, Felts and I showed that VLDL-TGFA, unlike FFA, are extensively taken up into adipose tissue,\(^24\) similar to earlier findings of Bragdon and Gordon for chylomicron-TGFA in rats.\(^{22}\) These observations suggested that the initial catabolism of chylomicron and VLDL-triglycerides follows similar pathways, although that of VLDL occurred at a slower rate. Figure 2 shows the major pathways of plasma free fatty acid and triglyceride transport and use, as understood in 1963.

The mechanism by which chylomicron-components other than triglycerides are removed from the blood remained a mystery, although early work by Max Biggs at UC Berkeley\(^27\) showed that dietary cholesterol is mainly deposited in the liver. Paul Nestel, Alice Bezman, and I found that a minor fraction of chylomicron triglycerides enters the liver without hydrolysis,\(^{28}\) and we postulated that chylomicron “skeletons” remaining after extrahepatic triglyceride-lipolysis might be taken up into hepatocytes by pinocytosis. Unlike chylomicron-TGFA, removal of the liver in dogs impaired clearance of cholesteryl esters from the blood, and in intact animals most of the cholesteryl esters rapidly appeared in the liver.\(^{29}\) We proposed that chylomicrons are removed from the blood in 2 steps: removal of much of component triglycerides into extrahepatic tissues via lipoprotein lipase action at the capillary endothelium followed by bulk removal of the remainder of the particle in the liver. As I noted in a 1965 review,\(^30\) the triglyceride-depleted chylomicron “skeleton” has “ready access” in the liver (via sinusoidal fenestrae) to the microvillus surface of hepatic parenchymal cells.

In the later 1960s, I initiated studies on the regulation of hepatic VLDL-triglyceride metabolism in dogs and humans with indwelling catheters in an hepatic vein (described below). In 1969, Emmett Bergman, on sabbatical leave from Cornell University, and I carried out experiments in unanesthetized dogs and sheep fitted with chronic indwelling vascular catheters in multiple sites (Bergman’s specialty) who received continuous infusions of triglyceride fatty acid and cholesteryl ester-labeled intestinal lymph. With this more physiological approach, we showed that removal of chylomicron-cholesteryl esters is highly efficient: about 10% was removed from hepatic sinusoids in a single pass, accounting for up to 90% of blood clearance, whereas triglyceride fatty acids were predominantly cleared in peripheral tissues and only about 10% in liver.\(^{31}\) In Australia, Trevor Redgrave had isolated chylomicron “remnants” from “supradiaphragmatic” rats, showing that these smaller particles are enriched in cholesteryl esters and depleted of triglycerides.\(^{32}\) Subsequently, in my laboratory, Ole Mjøs isolated rat VLDL-remnants as well as chylomicron remnants,\(^{33}\) showing that they share many properties (described below). Although the 2-step model became accepted for both chylomicrons and VLDL, only after Brown and Goldstein discovered receptor-mediated endocytosis in human fibroblasts\(^{34}\) were we able to demonstrate that this process is also used for the hepatic uptake of these lipoproteins (see below).

I continued work on FFA metabolism in dogs shortly after I came to UCSF and in 1959 initiated research on the role of FFA as an energy source in humans. This became possible because Comroe had created diagnostic facilities in the CVRI, including cardiac catheterization laboratories, which were also intended to support clinical research. Based on the hypothesis that removal of FFA from the blood might be

![Figure 2. This diagram, reproduced from reference,\(^26\) summarizes basic aspects of endogenous fatty acid transport in the blood, as delineated in the decade after 1953. Here are emphasized the major pathways of plasma FFA, derived from hydrolysis of triglycerides in adipocytes by a hormone-sensitive lipase. Bound to albumin (A), FFA, were rapidly removed into tissues,\(^12\) and shown to be a major energy source in the postabsorptive state for working skeletal muscle.\(^36\) In liver, fatty acids not used for oxidative metabolism were incorporated into triglycerides secreted into the blood in VLDL.\(^{24,25}\) On hydrolysis of VLDL in extrahepatic tissues by lipoprotein lipase at the surface of blood capillaries, product FFA were found to be extensively incorporated into adipose tissue triglycerides in the fed but not the fasted state,\(^24\) consistent with local activity of the enzyme. As shown, fatty acids thereby cycle between adipose tissue and the liver. Lipoprotein lipase–derived FFA also reenter the plasma directly.](http://atvb.ahajournals.org/)

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flow-limited, I postulated that FFA would be cleared mainly into working muscles and provide a major source of oxidative energy. By use of constant infusions of $^{14}$C-palmitate, Arnold Naimark, Christian Borchgrevink, and I were able to estimate the rate of FFA transport and formation of $^{14}$CO$_2$ in expired air in healthy men walking on a treadmill.\textsuperscript{35} We found that entry of FFA into the blood rapidly increases during exercise, and its oxidation provides about 50% of energy during moderate sustained leg exercise in the postabsorptive state. Alf Holmgren from the Karolinska Institute in Stockholm was working with others in the CVRI at this time, and I continued this and related work with him, Lars Carlson, and others during a sabbatical in Stockholm in 1962 to 1963. We found that infusing nicotinic acid prevented the augmented hydrolysis of triglycerides in adipose tissue during moderate exercise (presumably blocking the effect of adrenergic stimulation on lipolysis\textsuperscript{36}) and led to increased use of carbohydrate\textsuperscript{37}—an early in vivo demonstration of the glucose-fatty acid cycle in muscle. Back in the CVRI, Bengt Pernow, from the Karolinska, and I confirmed the initial studies of overall FFA clearance and oxidation by direct measures of FFA uptake and oxidation in the exercising leg.\textsuperscript{38} From measured exchanges of O$_2$ and CO$_2$ in the leg, we estimated that oxidation of local stores of triglycerides made an important contribution to oxidative metabolism, presaging work in many laboratories on the utilization of triglycerides stored within muscle cells.

In continuing work on FFA and triglyceride metabolism, I began to use hepatic vein catheterization to investigate quantitative aspects of the conversion of FFA to VLDL-triglycerides and transport rates of TGFA secreted from the liver in dogs and humans. The principal aim of this research was to provide a comprehensive picture of oxidative metabolism of hepatic fatty acids and the role of VLDL-triglyceride secretion in the regulation of plasma triglyceride concentrations. This work confirmed that plasma FFA are the predominant source of VLDL-TGFA in postabsorptive humans. By comparing normal with diabetic dogs,\textsuperscript{39} normotriglyceridemic subjects with those with primary hypertriglyceridemia\textsuperscript{40} and alterations produced by glucose-6-phosphatase deficiency (von Gierke disease)\textsuperscript{41} and infusion of ethanol,\textsuperscript{42} it became evident that increased secretion of VLDL-triglycerides derived from FFA could account some hypertriglyceridemic states (short-term insulin deficiency, glucose-6-phosphatase deficiency), but not others (long-term insulin deficiency, primary endogenously hypertriglyceridemia).\textsuperscript{1} By simultaneously measuring splanchnic fatty oxidation (to CO$_2$ and ketone bodies), we could estimate splanchnic (presumably hepatic) storage of fatty acids as well. Together with measurements of splanchnic balances of carbohydrates and, in some cases amino acid balances, this approach provided new insights into “caloric homeostasis.”\textsuperscript{43} For example, the reduction in hepatic oxidation of FFA by ethanol in subjects fasted for 3 days led to accumulation of triglycerides in the liver at a rate that could readily account for the rapid development of fatty liver in poorly nourished alcoholics.\textsuperscript{42} The status of the liver in glucose-6-phosphatase deficiency indicated a “fed” phenotype (in which oxidation of glycogen replaced that of FFA) with large increases in VLDL-triglyceride production. By contrast, peripheral tissues were confronted with hypoglycemia owing to reduced hepatic glucose production. They appeared “superfasted” with low-apolipoprotein lipase activities, contributing to the pronounced hypertriglyceridemia.\textsuperscript{41}

**UCSF and the Cardiovascular Research Institute. Phase II: Lipoprotein Biosynthesis and Catabolism**

John Kane joined my laboratory in 1967 as a postdoctoral fellow and became a doctoral candidate in Biochemistry. His topic was the structure of apolipoprotein B. Robert Hamilton, then a young cell biologist, joined me in 1970, initially on sabbatical leave from Vanderbilt, and Christopher Fielding, with a strong background in enzymology, and Phoebe Fielding came from the University of Chicago. At Comroe’s urging, our fledging group applied for and received an Arteriosclerosis SCOR award from the NHLI for a program on lipoprotein structure and function that, in addition to metabolism, involved protein chemistry, cell biology, and enzymology. This program continued for 25 years and had a profound effect on the work in my laboratory.

**Human Apolipoprotein Research**

Until the late 1960s, only the major apolipoproteins of HDL (apoA and LDL (apoB) were known, and these were poorly characterized. Additional apolipoproteins of low molecular weight, now known as apoCs, were identified in the laboratories of Peter Alapovic\textsuperscript{46} and Bernard and Virgie Shore.\textsuperscript{47} The Shores also discovered another protein of intermediate molecular weight (apoE) that was rich in arginine and exhibited 3 polymorphic forms.\textsuperscript{48} These discoveries ushered in a new era in lipoprotein research. In 1970 2 of the apoCs were isolated and characterized by Virgil Brown, Robert Levy, and Fredrickson.\textsuperscript{49} One of these (apoC-2) proved to be the “coprotein” found initially in plasma by Korn in his early work on lipoprotein lipase\textsuperscript{50,51} whereas the other (apoC-3) and a third small apolipoprotein (apoC-1) inhibited the effect of apoC-2 in vitro.\textsuperscript{51,52} With Moti Kashyap, we then found that the apoC transfer from HDL to triglyceride-rich lipoproteins (TRL) during postprandial lipemia and cycle back to HDL as lipemia clears.\textsuperscript{53}

Shortly thereafter, Kane and I found increased amounts of apoE among the soluble apoproteins of cholesterol-enriched β-VLDL and chylomicrons from patients with familial dysbetalipoproteinemia.\textsuperscript{54} These abnormal TRL, which exhibited properties of remnant lipoproteins, also had lower amounts of apoCs than the pre-β VLDL and postprandial chylomicrons.

\footnote{Although the pathogenesis of human hypertriglyceridemias has remained a controversial topic for many years,\textsuperscript{43} it is now recognized that increased VLDL-triglyceride levels that commonly accompany obesity and insulin resistance reflect combined effects of increased hepatic VLDL production and reduced clearance of VLDL-triglycerides. Recent work in the genomic era has shown that clearance of VLDL-triglycerides in peripheral tissues is subject to complex regulation by numerous proteins, mostly undiscovered in the early 1970s, including gene mutations and polymorphisms. One recent example from CVRI research is the observation among Asian populations with pronounced hypertriglyceridemia (above 500 mg/dL) that more than one-half have a specific coding polymorphism of apolipoprotein A-V.\textsuperscript{44}}
VLDL but contained much less unesterified cholesterol. VLDL from Golgi secretory vesicles that resembled plasma signatures were even more pronounced. Dysbetalipoproteinemia, in which we found that the remnant mass of remnant lipoproteins in familial homozygosity for one of these, apoE2, is associated with massive accumulation of remnant lipoproteins in familial dysbetalipoproteinemia, in which we found that the remnant signatures were even more pronounced.

Lipoprotein Biogenesis

At Vanderbilt, Hamilton had identified lipoproteins resembling VLDL by electron microscopy within membranes of the Golgi apparatus of in intact rat liver and a highly purified Golgi-rich fraction. In the early 1970s, we obtained nascent VLDL from Golgi secretory vesicles that resembled plasma VLDL but contained much less unesterified cholesterol. Golgi fractions obtained by another method, published in 1973, had a different morphology, and their content lipoproteins contained several-fold more unesterified cholesterol. It was only in the 1980s, after our autoradiographic studies with Albert Jones, described below, showed that 2 distinct lipoprotein-filled organelles are present at the apical pole of rat hepatocytes—Golgi secretory vesicles and multivesicular bodies containing endocytosed TRL-remnants—that we were able to show conclusively that endosomes massively contaminate Golgi fractions obtained by the widely used method. Notably, the membranes of multivesicular bodies, like plasma-membranes, were cholesterol-rich, whereas Golgi-membranes contained little unesterified cholesterol. Our definitive publication on the composition and other properties of nascent VLDL, published 20 years after we had initiated this work, also showed that no particles resembling LDL or HDL are evident in this immediate presecretory organelle. Golgi VLDL contained fewer C apoproteins than plasma VLDL and, accordingly, had slower electrophoretic mobility.

In a series of studies during the intervening years, we isolated and characterized lipoproteins secreted from perfused rat livers and obtained the first evidence, from localization of apoB by autoradiography within the secretory apparatus of hepatocytes, for a 2-step model of VLDL assembly within the endoplasmic reticulum. VLDL in liver perfusates contained more unesterified cholesterol than Golgi VLDL, presumably derived from erythrocytes in the medium. Like Golgi VLDL, perfuse VLDL were enriched in phospholipids and contained fewer C apoproteins than plasma VLDL. HDL were also found in perfusates, but they were discoidal when esterification of cholesterol (by lecithin-cholesterol acyltransferase [LCAT]) was inhibited. We found similar discs in the perfusate of rats in which VLDL secretion was abolished by treatment with orotic acid. Given the lack of evidence for HDL particles within the secretory pathway, we proposed that discoidal HDL are nascent particles that are formed extracellularly. Christopher Fielding showed that the discoidal HDL are excellent substrates for LCAT, which converted them to spherical HDL containing cholesteryl esters, resembling mature plasma HDL. We thus proposed that the nascent discs are the immediate precursors of mature HDL. Our identification of unesterified cholesterol-rich vesicular lipoproteins in patients and rats with cholestasis and the “discification” of cholesterol-poor phospholipid vesicles by apolipoproteins including apoA-I provided a theoretical framework for nascent HDL formation. Together with our studies and those of others on chylomicron secretion in rat intestinal lymph, a general framework for lipoprotein biogenesis emerged a quarter of a century ago in which the liver and intestine secrete nascent TRL, whereas HDL are formed extracellularly from polar lipids derived from TRL or cell membranes.

Receptor-Mediated Catabolism of Triglyceride-Rich Lipoproteins and LDL

The emergence of the LDL receptor pathway in cultured fibroblasts provided the framework to investigate the mechanism of TRL-remnant catabolism in the liver. In the late 1970s, Eberhard Windler, Yu-sheng Chao, and I found that rat apoE stimulates the uptake of chylomicrons in perfused livers, whereas each of the C apoproteins inhibits this effect, consistent with the alterations of apoproteins that we had previously found in chylomicron remnants. In estradiol-treated rats, known to exhibit profound hypolipi-
demia, we showed greatly accelerated catabolism of radioiodinated human LDL and HDL containing apoE, both in vivo and in perfused livers.75 Concurrent experiment in Brown and Goldstein’s laboratory demonstrated hepatic cell membranes from such rats exhibit a specific binding site for LDL.76 In a collaborative study, our two laboratories showed that liver membranes of treated rats exhibit a 10-fold increase in specific binding of lipoproteins containing apoB or apoE, but not HDL lacking these proteins—properties resembling that of the LDL receptor in cultured cells.77 We also showed that treatment with ethinyl estradiol dramatically increased the uptake of discoidal complexes of phosphatidyl choline with rat or human apoE into perfused rat livers.78 This was also the case for complexes from subjects homozygous for 2 of the 3 isoforms of human apo (E3 and E4), but not for the third, apoE2 from patients with familial dysbetalipoproteinemia, suggesting that defective binding of this isoform of apoE to an hepatic receptor with the characteristics of the LDL receptor is the cause of this disorder.79 A study involving 4 laboratories confirmed that apoE2 from most patients exhibits very low affinity for the LDL receptor.79 ApoE2 from a few affected patients, however, exhibited increased uptake into livers of estradiol-treated rats and close to normal binding to liver membranes from estradiol-treated rats as well as the LDL receptor in human fibroblasts, indicating genetic heterogeneity in this disorder and complexity in its pathogenesis.79 We confirmed this observation in a kindred with dominantly transmitted dysbetalipoproteinemia associated with an apparently mutant form of apoE3.80 Robert Mahlley’s group, who demonstrated that cysteine-arginine interchanges at 2 sites underlie the 3 common polymorphisms of human apoE, then demonstrated a distinct cysteine-arginine intercalage at position 142 in the receptor-binding domain of apoE in affected members of this kindred. Their studies of the functional characteristics of this mutant apoE3 as compared with that of apoE2 underlying the common recessive form of the disease demonstrated a combination of abnormalities that could plausibly account for dominant expression.81

In the early 1980s, I initiated studies of the fate of lipoproteins containing apoB and apoE in the liver. With Albert Jones, we used autoradiography to show that the intracellular pathway followed by LDL in hepatocytes of estradiol-treated rats via coated pits on the basolateral surface, sequentially to basolateral endosomes, multivesicular bodies, and lysosomes is consistent with that for receptor-mediated uptake and catabolism.82 This was followed by studies showing the same cellular itinerary for remnants of chylomicrons and VLDL in normal as well as estradiol-treated rats83 and by the isolation and characterization of early and late endosomes and a receptor-recycling compartment from rat livers.63,84 This work showed clearly that LDL and chylomicron and VLDL-remnants follow a common postendocytic pathway leading to lysosomal catabolism in hepatocytes.

I also initiated studies of hepatic lipoprotein catabolism in Watanabe hereditary hyperlipidemic (WHHL) rabbits, homozygous for a mutant LDL receptor that is receptor-negative for LDL (but not definitively for cholesterol-rich VLDL containing apoE). These animals are, however, hypertriglyceridemic as well as hypercholesterolemic and we found, together with Brown and Goldstein, that their VLDL (containing apoB-100 and rich in apoE), resemble remnant particles.85 By contrast, levels of apoB-48 in TRL were low. We then showed that the clearance of chylomicron remnants by the liver is unimpaired.86 This observation, together with results of binding studies to liver membranes, which showed that the WHHL mutation in the LDL receptor abolished the normal high-affinity binding of chylomicron remnants,86 led us to propose that the liver possesses a distinct receptor that, in addition to the LDL receptor, mediates the uptake of chylomicron remnants. We also found that secretion of apoB-100 from perfused livers of WHHL rabbits is not increased and occurs entirely in VLDL.87 With Nobuhiro Yamada and David Shames, I then undertook of series of studies on the metabolism of lipoproteins containing apoB-100 in these animals, initially confirming the observation of Toru Kita, David Billheimer, Brown, and Goldstein that formation of LDL from VLDL is increased.88 As in normal rabbits, however, conversion of VLDL particles lacking apoE to intermediate density lipoproteins (IDL) and LDL was greater than that of VLDL particles containing apoE.89 Furthermore, clearance of large VLDL particles containing apoE from the blood was rapid, comparable to that of chylomicron remnants.90 The latter observation resembled an earlier result in humans obtained with Anton Stalenhoef: large chylomicron and VLDL particles (from subject with lipoprotein lipase deficiency) were also cleared rapidly and at similar rates from the blood of healthy humans.91 These observations are consistent with the hypothesis that a hepatic receptor distinct from a the LDL receptor may mediate not only the uptake of chylomicron remnants but also that of remnants of hepatogenous TRL of sufficient size.

The discovery of a second member of the LDL receptor family by Joachim Herz92 that specifically binds lipoproteins enriched in apoE with high affinity (the LDL receptor-related protein [LRP]) raised the possibility that it could be a chylomicron remnant receptor. Henrik Lund, Hamilton, and I showed that LRP is highly expressed in endosome membranes of rat liver with a distribution comparable to that of the LDL receptor and other recycling receptors.93 Hamilton and I also observed that apoE decorates basolateral microvilli on rat hepatocytes and is rapidly released into the blood in a lipid-poor form by heparin and suramin, suggesting that it is bound to certain heparan sulfate proteoglycans present on the microvilli.94 Because we had found that LRP similarly decorates microvilli,93 we suggested that this apoE might enrich certain remnant lipoproteins, promoting their binding to LRP and facilitating subsequent endocytosis and catabolism.94 At the same time, with Stefan Jäckle we observed a striking delay in the rate of endocytosis of chylomicron remnants into rat hepatocytes in vivo as compared with apoE-rich β-VLDL from cholesterol-fed rabbits, although both were rapidly cleared by the liver.95 As we had not observed such a delay in estradiol-treated rats expressing high levels of the LDL receptor, we suggested that chylomicron remnants normally “bind to one or more
surface components on the sinusoidal plasma membrane of hepatocytes, such as heparan sulfate, hepatic lipase or LRP. Subsequently, with Shahida Shafi and Andre Bensadoun, we showed that removal of hepatic lipase from surfaces of rat liver by heparin or its inhibition with antibodies reduces the rate of uptake of chylomicron remnants but increases the rate at which the bound remnants are endocytosed, suggesting that the enzyme participates in the initial binding event. This observation, later confirmed in hepatic lipase–deficient mice, led us to propose that, lacking hepatic lipase, the remnants bind directly to endocytic receptors. Like apoE and LRP, hepatic lipase is now known to decorate hepatocytic endosome membranes from these rats. Notably, the hepatic uptake of apoE-enriched chylomicrons was reduced by only 30%, in support of the hypothesis that sites other than the LDL receptor or LRP participate in the initial binding event.

During the early 1990s, Herz and I attempted to assess the role of LRP in the hepatic catabolism of chylomicron remnants by use of the “receptor-associated protein” (RAP). RAP is a chaperone that binds with high affinity to LDL, preventing the binding and endocytosis of all known LRP-ligands, including apoE-enriched β-VLDL. Injection of RAP into rats, in amounts sufficient to prevent the hepatic uptake of another high-affinity ligand, (α-2 macroglobulin), abolished the endocytosis of apoE-enriched chylomicrons. However, it also substantially reduced hepatic uptake and endocytosis of human LDL in estradiol-treated rats, and we found that RAP does bind, albeit with low affinity, to the LDL receptor in endosome membranes from these rats. Notably, the hepatic uptake of apoE-enriched chylomicrons was reduced by only 30%, in support of the hypothesis that sites other than the LDL receptor or LRP participate in the initial binding event.

Herz and collaborators later showed that LRP is the only high-affinity ligand, (α-2 macroglobulin), abolished the endocytosis of apoE-enriched chylomicrons. However, it also substantially reduced hepatic uptake and endocytosis of human LDL in estradiol-treated rats, and we found that RAP does bind, albeit with low affinity, to the LDL receptor in endosome membranes from these rats. Notably, the hepatic uptake of apoE-enriched chylomicrons was reduced by only 30%, in support of the hypothesis that sites other than the LDL receptor or LRP participate in the initial binding event. Herz and collaborators later showed that LRP is the only high-affinity ligand.

The left portion shows that the receptor-mediated uptake and processing of these remnants, as understood in 1988, like that of LDL, follows the expected pathway of receptor-mediated endocytosis, with delivery via coated pits, into primary endosomes and thence, via sorting endosomes, into multivesicular bodies (late endosomes, MVB). Acidification, initiated in early endosomes, leads to dissociation of remnant particles from the membrane-bound receptors, which largely recycle to the cell surface. Acid hydrolases are present within the endosomes, but degradation of remnant components occurs only after transfer to lysosomes, where the internal pH is still lower. The right section of the diagram (2003) shows that remnants entering the space of Disse (SD) via sinusoidal fenestrae (arrow) bind initially to multiple components on the surface of microvilli. Prominent binding sites include heparan sulfate proteoglycans and heparan sulfate proteoglycan-bound apo E and hepatic lipase, as well as endocytic receptors (including the LRL receptor and LRP). ApoE may increase the affinity of remnants for LRP in particular and heparan sulfate can also bind LRP. Although it is evident that binding of remnants to microvilli precedes delivery of receptor-bound remnants to coated pits at the base of the villi, participation of these and possibly other components may be more complex than shown here (see text). The LDL receptor normally mediates the bulk of remnant-endocytosis, but LRP can compensate when it is deficient or downregulated. Modified from reference, with permission of the American Heart Association.

Recent work from the laboratory of Jeffrey Esko has shown that normal sulfation of membrane-bound heparan sulfate proteoglycans on mouse hepatocytes is required for normal removal of hepatogenous VLDL as well as chylomicron remnants in vivo. Previously, Alan Cooper’s laboratory showed that chylomicron remnants cluster with LRP together with syndecans on the surface of hepatocytes of LDL receptor–deficient mice. The clustering was eliminated by low concentrations of RAP. Recent reports from 2 laboratories suggest that the “lipolysis-stimulated receptor” discovered in Bernard Bihain’s laboratory has a role in the endocytosis of remnants of VLDL as well as chyomicrons postprandially.
bounds RAP, but with low affinity (=100-fold lower than that of LRP).117

Triglyceride-Rich Lipoproteins and Atherosclerotic Disease
During my investigative career, the importance of TRL in atherogenesis and atherosclerotic disease incidence has been more controversial than that of LDL. In his 1965 Lyman Duff Lecture, John Gofman, who originally proposed that S<sub>1</sub> 20–400 lipoproteins are more atherogenic than LDL (weight for weight),118 reported that prospective data from Framingham and Livermore failed to support this concept.119 (Footnote2)

For many years plasma triglycerides, as a surrogate for TRL, did not fare much better, owing in part to the log-linear inverse relationship between plasma triglycerides and HDL-cholesterol levels.120 More recently, a consensus has emerged that triglyceride concentrations do predict CHD incidence, independent of LDL and HDL-cholesterol.121 Evidence suggests that remnant-TRL are particularly atherogenic.122 Work in the UCSF SCOR has shown that TRL can be eluted with saline solution from carotid artery plaques obtained at endarterectomy in amounts proportional to ambient plasma TG concentration.123 These TRL are rich in apoE. In a prospective study of patients undergoing repeated coronary angiography during a clinical trial, we found increased levels of TRL-remnants and reduced levels of HDL-cholesterol to be independently associated with lesion progression and clinical events related to coronary artery disease.124 Neither plasma triglycerides nor LDL-cholesterol showed these associations.

Kane’s discovery of 2 forms of human apoB, showing that the intestine secretes a smaller form (B-48) than the liver (B-100),125 has made it possible to distinguish and quantify the origin of TRL in clinical research.126 The apoE-rich TRL that we eluted from carotid plaques were thus shown to be overwhelmingly of hepatic origin.123 During postprandial lipemia in healthy men, we found that 80% of the increment in TRL particle number is attributable to hepaticogenous TRL and only 20% to intestinal particles.127 Japanese colleagues, Elisa Campos, and I developed a clinically suitable immunochemical method to separate “remnant-like particles” from fasting and postprandial plasma.128 Quantification of these particles, which include all TRL containing apoB-48 and a subtraction of TRL containing apoB-100,129 has found considerable application in clinical studies. During the 1990s, my laboratory used these new methods in additional studies of plasma lipoprotein alterations during postprandial lipemia.130 This work and that of others has contributed to a resurgence of interest in the clinical significance of postprandial TRL in atherogenesis and atherosclerotic vascular disease. Recent studies suggest that postprandial rather than fasting plasma TG concentrations may better predict CHD events.131 After meals containing fat, preferential interaction of chylomircrons with lipoprotein lipase delays VLDL catabolism.130 This not only increases VLDL levels, but also leads to cholesteryl ester enrichment of VLDL owing to enhanced transfer of LCAT-derived cholesteryl esters from HDL via CETP.127,132 Thus, particles with properties of remnants in humans are more complex than those of rodents and other animals lacking the transfer process. Current evidence suggests that hepatic uptake and catabolism of LCAT-derived cholesteryl esters within human triglyceride-rich lipoproteins may normally constitute the major pathway for reverse cholesterol transport.

The fact that the concentration and properties of TRL are intimately tied to those of LDL and HDL makes it virtually impossible to determine the magnitude of the direct contribution of triglyceride-lipoproteins to atherogenesis and coronary heart disease. The same dilemma applies to evaluating the importance of the related alterations in the size and composition of LDL and HDL. Common interventions that reduce TRL levels, however, have salutary effects on the cholesterol-rich lipoproteins.

Concluding Remarks
Advances in research on the plasma lipoproteins and their role in lipid transport during the last half of the twentieth century are central to our understanding of the causes of atherosclerotic vascular disease. These advances have depended on new methodologies required to study macromolecules and macromolecular complexes, the availability and use of isotopic tracers, various cell biological approaches, and most recently the advent of concepts and methods of molecular biology and genetics. In my case, the ability to move repeatedly between studies involving healthy or diseased humans and experimental animals as these developments have occurred, and to work with collaborators of differing expertise and knowledge have been special gifts. Although the overall themes of lipid transport in lipoproteins emerged rapidly during this period, the detailed analysis of each step continues to yield new surprises and insights. In particular, in the era of genome-wide technologies the study of candidate gene mutations and polymorphisms remains fruitful.

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None.

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