Meeting Summary

Relationship of Hypertriglycerideridemia to Atherosclerosis

Kenneth Lippel, Herman Tyroler, Howard Eder, Antonio Gotto, Jr., and George Vahouny

On June 8–10, 1981, the Lipid Metabolism-Atherogenesis Branch of the National Heart, Lung, and Blood Institute sponsored a workshop entitled, "The Relationship of Hypertriglycerideridemia to Atherosclerosis," in Bethesda, Maryland.

The purpose of the workshop was to bring together a large number of investigators with primary interests in epidemiology, genetics, nutrition, pathology, and basic and clinical research to discuss recent advances in the knowledge of triglyceride metabolism and to summarize the relationships of the hypertriglycerideridemias to atherosclerosis. The agenda was planned by Drs. Elizabeth Barrett-Connor, Bryan Brewer, John Brunzell, Christopher Fielding, Scott Grundy, Kenneth Lippel, Arno Motulsky, and Donald Zilversmit. More than 80 scientists attended the workshop; 26 made formal presentations or served as panel discussants. The other attendees participated in the discussions and in the question and answer periods that followed each presentation. We present here a summary of the major presentations made at the workshop as well as a list of some questions that will likely be the basis for future research in triglyceride metabolism and in the relationships of the hypertriglycerideridemias to atherosclerosis. The summary is organized into three sections which correspond to the framework of the workshop.

Session on Epidemiology

The first day's session was chaired by Dr. Herman Tyroler. The epidemiologic presentations at the workshop consisted of systematic and critical reviews of published research on the association between triglycerides and ischemic heart disease (IHD) from substantive, methodologic, and conceptual perspectives. In addition, the results of presently unpublished findings from several population-based studies were reported. The information presented was evaluated, and future studies were considered.

Hypertriglycerideridemia: Overview

After opening remarks by Dr. Kenneth Lippel, Dr. Basil Rifkind presented an overview of the frequency and distribution of hypertriglycerideridemia in populations. Attention was called to problems in definition, measurement, and the requirement of fasting. The results of the large scale, North American Lipid Research Clinics Data Book were highlighted. Associations of triglyceride (TG) with sex and age, well known in general, were quantified (e.g., mean TG rose from approximately 55 to 155 mg/dl with age, with differences by sex). One of the major determinants of hypertriglycerideridemia in premenopausal women was oral contraceptive use. Less well-known characteristics and implications of the empirical distribution of fasting TG were discussed; age- and sex-specific 95th percentiles were much higher than standards previously employed. For example, the prevalence of Type IV hyperlipoproteinemia in the LRC Population Studies would be approximately 20% based on the older National Heart, Lung, and Blood Institute cutoff points, but less than 4% based on the empirical 95th LRC percentile use.
**Case Studies of Hypertriglyceridemia**

Dr. Charles Glueck reviewed case control studies, including those based on clinical studies of individual patients and their controls, family studies, angiographic studies of the presence and extent of angiographically determined coronary atherosclerosis, and the relation of triglycerides to occlusion of saphenous vein bypass vessels. Overall, most of the studies reviewed demonstrated a univariate association of coronary heart disease to triglycerides. Some, but not all studies, reported statistical control for total plasma cholesterol, but only a few reported statistical control for high density lipoprotein (HDL) cholesterol. In the instances where statistical analyses were carried out to control for other lipids, the results were inconsistent, with some studies disclosing stronger relationships of coronary heart disease with triglycerides, others with total cholesterol. Few of the case control studies reviewed carried out systematic multivariate analyses to appraise the independent risk factor status of triglycerides.

**Cohort Studies of Hypertriglyceridemia**

Dr. Gerardo Heiss reviewed the published cohort studies. In contrast to the case control studies, in which triglyceride levels of patients with clinically manifest disease were compared with putatively disease-free controls, the cohort studies measured triglyceride levels in individuals clinically free of coronary heart disease. The relationship of these triglyceride levels to risk of subsequent development and manifestation of disease was then assessed. The workshop participants emphasized the importance and relevance of the ability of this research design to distinguish between antecedent and consequent for the problem at hand, because hypertriglyceridemia following myocardial infarction (MI) has been documented.

Dr. Heiss reviewed 12 cohort studies published since 1965, which were carried out in populations residing in Sweden (Goteborg and Stockholm), Norway (Tromso), Japan, Hawaii, and the United States mainland. Of 11 univariable analyses, seven studies disclosed a positive association between baseline TG and the incidence of IHD. When multivariable analyses were performed, adjusting TG levels for covariates, the majority of the studies reported no residual association between TG and IHD incidence.

The following four studies of the relationship of TG levels at intake to IHD, or to total cardiovascular mortality (CVM), were reviewed; studies from Finland, Evans County, the U.S. LRC Mortality Follow-Up Study, and the Coronary Drug Project. Only one study showed an association of CVM with TG; this represented an incomplete analysis, omitting blood pressure.

Despite limited evidence of an independent role of TG in IHD incidence and CVM, based on epidemiologic cohort data, attention was called to the central role of VLDL-TG in lipid metabolism: atherogenic LDL is largely a product of VLDL catabolism; antiatherogenic HDL is inversely correlated with TG. The techniques of statistical control used in the nonexperimental, observational epidemiologic studies might not be adequate to identify TG links in a causal pathogenetic pathway.

Dr. Lars Carlson presented a review of 20 years' experience in the study of the TG-IHD relationship in Stockholm. Three types of studies were reviewed: 1) case control studies of myocardial infarction (MI) survivors, which disclosed a fivefold excess of hypercholesterolemia and hypertriglyceridemia (greater than or equal to the 95th percentile) and accompanying elevations of LDL and VLDL in cases of IHD compared to controls; 2) cross-sectional studies, which disclosed an association of hypercholesterolemia and hypertriglyceridemia with positive exercise electrocardiograms in symptom-free men; and 3) cohort studies, which disclosed an association of TG with fatal and nonfatal IHD in men and women. The TG association with incidence of IHD was statistically significant after controlling for age, systolic blood pressure, smoking, weight/height index, hemoglobin, and erythrocyte sedimentation rate by multiple logistic analysis. Total cholesterol was not a statistically significant predictor of mortality, and HDL-cholesterol was not included in this model.

Dr. Stephen Hulley reviewed the analyses of the Western Collaborative Group Studies (WCGS). The incidence of IHD was twice as high in the upper than in the lower TG strata in univariate analysis. In univariate logistic analysis, the standardized odds ratio was 1.36 for TG and 1.67 for total cholesterol, each statistically significant (p < 0.001). In bivariate logistic analysis of IHD in relation to TG and total cholesterol, the standardized odds ratio for cholesterol remained statistically significant and was little changed at 1.60 by inclusion of TG in the model. In contrast, the standardized odds ratio for TG was reduced to 1.13 and was not statistically significant when cholesterol was considered simultaneously. The standardized odds ratio for TG in relation to IHD was slightly less than one and not statistically significant upon multivariate analysis, with TG, total cholesterol, HDL-cholesterol, and body mass index in the model. In this multiple logistic regression model, the standardized odds ratio for total cholesterol remained elevated (1.55) and statistically significant. Dr. Hulley reviewed the position of his recent article in the New England
that the total body of available evidence — biologic, statistical, and epidemiologic — argued against a causal role for TG in the etiology of IHD.

**Multivariate Analyses**

Dr. Robert Abbott reviewed the properties of multivariate analyses relevant to the association of TG with IHD. In addition to the general limitations of any statistical method in drawing causal inferences from observational, nonexperimental research, particular attention was called to the effects of correlation between two independent variables (such as HDL cholesterol and TG) in reducing the predictiveness of either one for a dependent variable (such as IHD incidence rate), while controlling for the effect of the other independent variable. This property of reduced predictiveness for any given sample size was schematically illustrated by considering the joint distribution of the two independent variables as the floor from which columns rose to support the dependent variable. If the two independent variables were not correlated, the base would be broad and stable; if correlated, the base would be narrow and the support unstable. The resulting statistical effect is that estimates of the magnitude of the coefficient expressing the relation between one of the independent variables (e.g., TG or HDL cholesterol) and the dependent variable (e.g., IHD incidence rate) would be "unstable." Thus, assessment of the independent contribution of a risk factor in multivariate analysis, when it is in a model containing other variables with which it is highly correlated, is problematic.

**Measurement of Triglycerides**

Dr. Elizabeth Barrett-Connor reviewed the difficulties in measuring TG in epidemiologic studies. Laboratory and intraindividual sources of variability, each considerable, contribute to misclassification and the potential for bias in assessing the relation of TG to IHD. The estimates of the relative risk of IHD associated with hypertriglyceridemia were cited as being in the range of 1.3 to 3.6 on univariate analysis, with evidence of a dose-response relation. Reviewing other criteria for a causal relation, Dr. Barrett-Connor noted the consistency of the finding across studies (in univariate analysis) and the appropriate time sequence of hypertriglyceridemia preceding IHD (demonstrated in the cohort studies). The major outstanding question was the independent risk factor status of TG.

**Future Research**

Panel discussants Drs. Herman Tyroler, Lewis Kuller, Lars Carlson, and Stephen Hulley identified a need for the following studies: epidemiologic cohort studies of the IHD sequelae of different TG and lipoprotein levels and composition; IHD case control studies to investigate the heterogeneity of hypertriglyceridemia; analysis from clinical trials of the (secondary) effects on triglycerides of interventions designed for other purposes; family aggregation and genetic studies of hypertriglyceridemia and IHD; studies of the determinants of the distribution of TG levels in populations; studies of the determinants of the markedly lower TG levels observed in several U.S. black male populations which have IHD rates lower than their white peers.

In summary, the evidence from most epidemiologic studies identified an association between IHD and TG in a univariable mode of analysis. The participants identified only one prospective study in which this association persisted after statistical control for other plasma lipids; this study, however, did not include HDL cholesterol among the covariates of TG. Thus, the extant epidemiologic evidence does not support an independent association between elevated TG and subsequent development of IHD. However, the participants acknowledged the central role of TG and its transport in chylomicrons, VLDL, and their remnants, for lipid metabolism and possible atherogenic mechanisms. Therefore, the failure to demonstrate independent association between TG and IHD in population-based observational studies should not discourage the efforts to provide insight at molecular and pathophysiologic levels.

**Session on Metabolism of Triglyceride-Rich Particles**

The second day’s session, chaired by Dr. Howard Eder, consisted of reviews of published research and unpublished data related to the metabolism of triglyceride-rich particles (chylomicrons, VLDL, and their remnants) and their possible relationships to atherogenesis. The role of the LCAT enzyme in hypertriglyceridemia was also discussed. The information was evaluated and future studies were considered.

**Overview**

Dr. Richard Havel presented an overview of the metabolism of triglyceride-rich particles. He noted that triglyceride-rich particles separated
by ultracentrifugation include intestinal chylomicrons of various sizes, chylomicron remnants, and hepatic VLDL and their remnants; they are heterogeneous in their metabolic activities as well as in their interactions with cells. Their heterogeneity can be identified in two ways. One is the recently described difference in the electrophoretic mobility on SDS gels between the apo B of intestinal origin and the apo B of hepatic origin. Secondly, partially catalyzed particles can be separated from native particles by electrophoresis on the basis of altered surface change.

The surface proteins of newly secreted intestinal chylomicrons are apoaproteins B, A-1, A-2, and A-4. The apo B is the lower molecular weight particle (B<sub>100</sub>). Upon entry into the lymph and then into the plasma, chylomicrons acquire C-apoproteins and apo E from HDL by exchange with phospholipid. The latter apoproteins are not synthesized at any appreciable extent in the intestine. The modified particles, having acquired apo C-2, an activator of lipoprotein lipase, then interact with lipoprotein lipase, and the triglyceride component is rapidly hydrolyzed. The resulting particles are enriched in cholesterol ester and modified at the surface, with most of the C-apoproteins and A-apoproteins returning to HDL. The resulting chylomicron remnants then interact with receptors on hepatic parenchymal cells and are metabolized.

The apoproteins are major determinants of the metabolism of the chylomicron remnant particles. Apo E is recognized by what appears to be a specific hepatic receptor for apo E (as distinct from the B,E receptors). The apo B<sub>48</sub> is not recognized by the B,E receptor so that the E is the determinant of recognition (unless there is a specific B<sub>48</sub> receptor). C-apoproteins, in addition to activating lipoprotein lipase, also modulate the uptake of lipoproteins by the liver and prevent the premature uptake of chylomicrons.

In both man and rats, LDL is apparently not formed from chylomicrons as evidenced by the rapid removal of B<sub>48</sub>-containing lipoproteins and the failure to recover B<sub>48</sub> in LDL.

However, patients with severe hyperlipemia (TG greater than 1000 mg/dl), with circulating chylomicrons, do have lipoproteins containing B<sub>48</sub> in their plasma; B<sub>48</sub> is also found in plasma of patients with familial dysbetalipoproteinemia (Type III), indicating a defect in chylomicron metabolism. VLDL secreted by the liver differ from triglyceride-rich particles secreted by the intestine in that the former contain no A apoproteins, but do contain apo B, C, and E which are not synthesized by the intestine. Only the high molecular weight apo B (B<sub>100</sub>) is present in hepatic VLDL (in humans and rabbits; however, in rats the liver secretes VLDL containing B<sub>100</sub> and some B<sub>48</sub>). Hydrolysis of triglyceride is slower in VLDL and the turnover rate is slower than that of chylomicrons.

The remnant particle formed from VLDL (IDL) is metabolized differently from the chylomicron. Conversion of IDL to LDL probably occurs in the liver through the action of hepatic triglyceride lipase. The B<sub>100</sub> is recognized by B,E receptors (which may not be active in the adult animal). In humans only a fraction of the IDL is removed by the liver and the remainder is converted to LDL; in rats most of the IDL is removed by the liver, with only a small portion being converted to LDL.

Dr. Havel also discussed cholesteryl ester formation and transport and the importance of transfer of cholesteryl esters to remnants that are removed by the liver where they can be converted to bile acids. He discussed the work of Utermann and of Zannis and Breslow on the polymorphism of apo E and noted that the liver exhibited higher receptor activity for certain of the isoforms, and lower affinity for the isoforms present in Type III hyperlipoproteinemia. While Type III patients are homozygous for the beta IV allele, heterozygotes containing this allele may also have mild impairment of remnant removal.

In response to a question about immunologic differences between B<sub>100</sub> and B<sub>48</sub>, it was noted that with polyclonal antibodies both proteins are recognized, but different antisera vary in their specificity for each protein.

**Metabolism of Chylomicron Remnants**

Dr. John Dietschy discussed the metabolism of chylomicron remnants and its importance in chylomicron metabolism. He noted that it is via the chylomicrons that cholesterol of intestinal origin is delivered to the liver where it can be excreted.

In the studies described, turnover was measured by constant infusion of labeled lipoprotein to achieve a constant plasma level from which the rate of lipoprotein clearance could be readily calculated. In the rat, chylomicrons are cleared at a rate of 7000 μL of plasma per hour, LDL at 824 μL per hour, and HDL at 644 μL per hour. Human HDL cleared at an even slower rate.

To determine the role of the liver in lipoprotein removal, three-fourths of the liver was removed. Chylomicron removal was decreased by 75%; LDL clearance, by 27%; and HDL clearance, by 19%. From these data it was concluded that essentially 100% of the chylomicrons, 45% of the LDL, and about 25% of the HDL is cleared by the liver. A similar conclusion was reached by use of sucrose-labeled LDL in a constant infusion.

Removal by the liver was measured by infusion of various lipoproteins for 40 hours and measurement of changes in the rate of cholesterol synthesis. Infusion of chylomicrons caused a marked decrease in cholesterol synthesis by the liver;
Role of the Liver in Cholesterol Synthesis

Dr. Dietschy discussed the importance of the liver as a site of cholesterol synthesis in the intact animal. He noted that with use of $^3$H$_2$O as a precursor for cholesterol, the estimate of peripheral synthesis is higher than when acetate or octanoate is used. With $^3$H$_2$O it was estimated that 50% of cholesterol synthesis occurred in the liver and the remainder in peripheral tissues including the adrenal, ovary, intestines, and skin. In the rabbit, 17% of cholesterol synthesis was found to occur in the liver, and in the squirrel monkey hepatic synthesis was 45% of total synthesis. In humans, hepatic synthesis can only be estimated and is probably relatively low.

Since adaptation to increased cholesterol intake is partly accomplished by reducing cholesterol synthesis in the liver, the rat, with a high rate of hepatic synthesis, can readily adapt. However, the rabbit has a low rate of hepatic synthesis and only a limited capacity to make bile acids, so that plasma cholesterol rises promptly with cholesterol feeding. Man has a limited capacity to absorb cholesterol and is thus protected against excess dietary cholesterol. However, there is an additional homeostatic mechanism in that loss of cholesterol from the liver (as after administration of cholestyramine) results in an increased number of receptor sites so that uptake of cholesterol by the liver increases.

Chylomicron Metabolism in Humans

Dr. Ernst Schaefer discussed chylomicron metabolism in humans. He presented data on thoracic duct lymph chylomicrons and cautioned that they may differ substantially from mesenteric lymph chylomicrons, which cannot be obtained in human subjects.

The apoprotein composition of human thoracic duct chylomicrons is approximately: apo A-1, 9%; A-2, 4.5%; B, 12%; C-2, 13%; C-3, 28%; and A-4+albumin+E, 25%. Although the content of C apoproteins is high, they are not synthesized in the intestine, whereas apo B, A-1, A-2, and A-4 present in lymph are synthesized in the intestine. The apo B consisted of several bands, probably from admixture of plasma with lymph.

When $^{125}$I-labeled chylomicrons were injected into human subjects, radioactivity disappeared rapidly from the chylomicron fraction, and appeared in VLDL, IDL, and LDL. The apo A-1 and A-2 ends up in HDL and then turns over at the same rate as the apoproteins present in HDL. About 10% of the apo B was found in LDL, but it was not known which of the apo B fractions contained the radioactivity. C-apoproteins transferred to VLDL, LDL, and IDL, but later returned to the chylomicrons.

Apoprotein output in human thoracic duct lymph was quantitated. The lymph contained some LDL and HDL in addition to chylomicrons. The HDL was chiefly HDL$_{2a}$, with little HDL$_{3}$. Of the total A-1 in the lymph, about 16% was in the d < 1.006 g/ml fraction, 80% in HDL, and the remainder in the d > 1.21 g/ml fraction. A-2 had a similar distribution.

Apo A-1 and A-2 output per 24 hours was measured and found to exceed predicted synthesis, suggesting that there was considerable admixture with plasma HDL. It was estimated that of the A-1 produced, 25% enters with intestinal chylomicrons, 25% with intestinal HDL, and 50% comes from the liver.

Dr. Schaefer closed his presentation by noting that chylomicrons (present in patients with Types I and V hyperlipoproteinemia) are not associated with extensive atherosclerosis, whereas chylomicron remnants present in patients with Type III hyperlipoproteinemia may be very atherogenic.

Mechanism of Hypertriglyceridemia

Dr. Grundy discussed the mechanism of hypertriglyceridemia, noting that it could be due to either overproduction or defective catabolism (or both). He described the use of Zech's kinetic model to study the production and metabolism of VLDL triglyceride (using tritiated glycerol). This model uses multicompartamental analysis to analyze the curve of VLDL radioactivity over time. This curve is divided into three components: 1) the initial rise in activity; 2) early fast decay; and 3) late slow decay, the tail. This method was used to study patients with maturity onset diabetes and hypertriglyceridemia. These patients had a high production rate, which
decreased after treatment with insulin, but insulin did not appreciably change the fractional catabolic rate. Patients with primary hypertriglyceridemia and obesity were also studied before and after weight reduction. Fractional catabolic rates were essentially unchanged and it was concluded that the hypertriglyceridemia was caused primarily by overproduction, but that this may be associated with decreased clearance which exacerbated the hypertriglyceridemia.

In the discussion, Dr. Grundy noted that some very obese patients did not have hypertriglyceridemia despite overproduction, but they did have increased clearance. He also stated that obese patients use the slow pathway to a greater extent.

Metabolism of Apo B

Dr. Barry Lewis discussed the metabolism of apo B. He demonstrated correlation between VLDL-TG concentration and the rate of synthesis of VLDL apo B. In patients with familial combined hyperlipidemia, there was considerable overproduction of VLDL apo B, whereas this was not evident in patients with familial hypertriglyceridemia who had impaired removal of VLDL apo B.

In earlier studies of the conversion of VLDL apo B to LDL apo B, the d = 1.006-1.019 g/ml fraction was considered to be LDL; more than 90% of VLDL apo B appeared in this fraction in normal subjects, although in patients with hypertriglyceridemia, less of the VLDL apo B was recovered in this fraction. In more recent studies, the d = 1.006-1.019 g/ml (IDL) and d = 1.019-1.063 g/ml (LDL) fractions have been separated; about half of VLDL apo B appeared in the LDL fraction.

This question of conversion of VLDL apo B to LDL apo B has also been studied by the more direct method of measuring arterial-venous differences in lipoprotein concentrations across the splanchnic circulation by catheterization of the hepatic vein and the coeliac axis. These studies indicated secretion of VLDL across the splanchnic circulation and extraction of IDL. About 55% of the IDL taken up by the splanchnic bed appeared in the hepatic vein as LDL, confirming the turnover data. In hypertriglyceridemic subjects there was increased secretion of VLDL, but no increase in IDL removal, suggesting the possibility of VLDL metabolism in peripheral tissues. In Type III patients, conversion of remnants (IDL) to LDL was greatly decreased.

Dr. Lewis also described studies in which labeled homologous VLDL was injected into subjects 24 hours before arterial surgery. Pieces of artery were extracted and lipoproteins extracted and separated by ultracentrifugation. The greatest flux was for LDL; however, apo B in VLDL and IDL was also transferred to the arterial wall, but at a lower rate than LDL.

In summary, Dr. Lewis listed his three major conclusions:

1. VLDL concentration is largely determined by the rate of synthesis, but especially in certain individuals, differences in catabolism of apo B are an important determinant.

2. The rate of VLDL catabolism is one determinant of the concentration of HDL.

3. Triglyceride-rich lipoproteins do enter the arterial wall in vivo.

Triglyceride-Rich Lipoproteins

Dr. Zilversmit discussed how triglyceride-rich lipoproteins might cause atherosclerosis. He suggested two possible mechanisms: 1) chylomicron remnants in the plasma may be atherogenic; and 2) the metabolism of the chylomicrons on arterial walls may produce atherogenic particles. Chylomicrons (and VLDL) can interact with endothelial cells or elements of the subendothelial layers (e.g., smooth muscle cells and lipoprotein lipase present in these tissues could interact with these particles, leading to particles that could be internalized by apo B receptors). He described experiments indicating that cholesterol-rich chylomicron remnants are present in the plasma of cholesterol-fed rabbits. After the feeding of cholesterol to rabbits, their VLDL rose. To determine whether the VLDL was of hepatic origin, retinol was fed; retinyl ester accumulated in the plasma of cholesterol-fed animals, but not in control rabbits. Dr. Zilversmit noted that recent studies have shown that retinyl ester can transfer to other lipoproteins which may complicate the use of this technique. Several interpretations of these data are possible. One is that the system for remnant removal is quickly saturated. A second possibility is that cholesterol is removed by the liver, VLDL secretion is stimulated, and these particles compete with particles of intestinal origin for removal.

In another series of experiments, the lipid and the apoproteins were both labeled; the uptakes of both were identical, indicating that the entire lipoprotein was taken up by the artery. This was true for VLDL, remnants, and LDL. However, the uptake of LDL was three times higher than that of VLDL. When other proteins such as HDL or albumin were used, it was found that the smaller the particle, the higher the uptake by the arterial wall.

During the discussion, Dr. Havel reported that the cholesterol-rich particles were associated
with particles containing almost exclusively apo B₄₁₂, suggesting that they are of hepatic, not intestinal, origin. Dr. Eder reported that the cholesterol-fed diabetic rat shows marked hypercholesterolemia and that chylomicrons and remnant particles contain both large and small B-apoproteins, suggesting contributions by both liver and intestine. The Zilversmit hypothesis was questioned on the basis of epidemiologic studies which show that in countries where fat intake is high (e.g., Crete and Greece) there is little atherosclerosis. Dr. Fisher described studies of apo B synthesis, with labeled leucine used as a precursor. Kinetic analysis of the data suggested direct input of apo B into IDL without prior incorporation into VLDL. In the subsequent discussion it was reported that there could be dissociation between apo B removal and triglyceride removal in some patients, with apo B removed more rapidly than triglycerides. Dr. Esko Nikkilä described studies in rats treated with antisera to hepatic triglyceride lipase (HTGL). He found no difference in removal of chylomicrons or chylomicron remnants between the treated and untreated rats. Dr. Lewis noted that in patients with Type I hyperlipoproteinemia lacking lipoprotein lipase (LPL), heparin caused release of HTGL and HDL phospholipid decreased.

VDL Conversion to LDL

Dr. Shlomo Eisenberg discussed studies of the transfer of apo B from VLDL to IDL and LDL. He noted that previous studies indicated that one particle of VLDL was converted to one particle of IDL, which in turn was converted to one particle of LDL. He then described in vitro studies with VLDL and LPL, which led to the production of LDL similar to native LDL but with a low triglyceride content. He also described studies indicating how the excess cholesteryl ester in VLDL is lost during conversion to LDL. The initial LDL particle is enriched in triglyceride with loss of cholesteryl ester. It also contains apo C and apo A-1. Further action of LPL on this particle results in loss of triglyceride and a smaller particle rich in cholesteryl ester, but containing less cholesteryl ester than the precursor LDL. The surface components lost during this process form HDL₃, which is then converted to a particle of density similar to that of HDL₂ by addition of surface components from VLDL. The cholesteryl ester of HDL₂ is transferred to VLDL and the cycle is repeated.

Dr. George Steiner discussed heterogeneity in VLDL production and catabolism. Using labeled S₃, 20-400 lipoproteins, he found a precursor-product relationship with S₁, 12-20 lipoproteins; however, measurement of turnover indicated that not all of the S₃, 20-400 fractions appeared in the S₁, 12-20 fractions. All of the apo B in the IDL fraction (S₁, 12-60) came from VLDL (S₃, 60-400) and there was no loss of apo B in that step. However, in patients with Type III hyperlipoproteinemia, there was independent production of IDL.

Simultaneous studies were done with iodinated VLDL and labeled glycerol. The disappearance of VLDL and appearance of IDL indicated a precursor-product relationship for apo B but a dynamic state with triglyceride entering and leaving the particle at various stages of its metabolism. The mechanism of independent triglyceride entry into the smaller particles is not known and various hypotheses were suggested.

Delivery of Cholesteryl Ester to Smooth Muscle Cells

Dr. Edwin Bierman discussed delivery of cholesteryl ester to cultured smooth muscle cells by remnant lipoproteins. To test the Zilversmit hypothesis, uptake of chylomicron remnants by cultured human arterial cells was studied. Chylomicrons were isolated from a hypertriglyceridemic donor fed cream, and remnants were produced by incubation of these chylomicrons with post-heparin human plasma. The remnants were as effective as LDL in delivering cholesterol to the cells and down-regulating the LDL receptor. When remnants of different sizes were compared, it was found that the small remnants were more effective in delivering cholesterol to cells. Remnants were as effective as LDL in increasing cellular cholesterol content when an equal amount of cholesterol was given either as remnant or as LDL. Calculation of the apo B content of the various particles revealed that when the number of remnant particles was equal to the number of LDL particles, the effect was similar and perhaps remnants were even more effective than LDL in delivering cholesterol to the cells.

Acetylated LDL was taken up by human monocyte derived macrophages and this uptake could be inhibited by excess unlabeled acetyl LDL, but not by chylomicron remnants. However, there was appreciable uptake of remnants which was competitively inhibited by excess remnants. Normal LDL was also taken up and degraded, and chylomicron remnants competed very effectively for LDL uptake and degradation. It was concluded from these studies that chylomicron remnants cause cholesterol accumulation in arterial smooth muscle cells and are therefore potentially atherogenic.

In the discussion, factors other than size of the remnant particles were considered as the cause of the differences in uptake of large and small remnant particles. It was also noted that in vivo, platelet-derived growth factor may be present so
that the number of active receptors might be increased despite the high concentrations of lipoproteins to which these cells are exposed in vitro. There was also discussion as to whether the uptake demonstrated in these studies was receptor mediated, since the experiments were performed at saturating concentrations of the lipoproteins. Dr. Zilversmit noted that the process described by Dr. Bierman may not hold in the intact animal.

Uptake of Beta-VLDL Particles

Dr. Thomas Bersot then discussed mouse peritoneal macrophage uptake of $d < 1.006$ g/ml particles (beta-VLDL) obtained from either cholesterol-fed humans or cholesterol-fed rhesus monkeys. Foam cells were produced by incubation of the macrophages with the beta-VLDL particles. Cholesteryl ester accumulation was estimated by determining acyl-CoA cholesterol acyl transferase (ACAT) activity by measurement of incorporation of $^{14}$C oleate into cholesteryl esters after incubation with various lipoproteins. Only the beta-VLDL caused cholesteryl accumulation in macrophages. Humans were fed 2 g of cholesterol per day for 3 weeks. In these subjects the slow migrating, cholesterol-enriched VLDL (beta VLDL) markedly stimulated cholesteryl ester accumulation, which occurred despite the fact that there was only a modest increase in plasma cholesterol following the cholesterol feeding. The property of beta-VLDL that causes this accumulation of cholesteryl ester is not known. However, there does appear to be a correlation with the cholesteryl-to-protein ratio of the particle (i.e., particles with a ratio greater than 1 stimulate cholesteryl uptake).

Dr. Bierman questioned the relevance of the mouse peritoneal macrophage to human atherosclerosis, noting that he was unable to demonstrate cholesteryl ester accumulation in cultured arterial smooth muscle cells with plasma obtained after a cholesterol-rich fat meal. In further discussion, Dr. Bersot revealed that the beta-VLDL contained 50% to 55% triglyceride and 15% to 25% cholesteryl ester. It is enriched in apo E and probably in the apo B^ of intestinal origin.

Lecithin Cholesterol Acyl Transferase Activity

The final paper of the session was a discussion of lecithin cholesterol acyl transferase (LCAT) activity in hypertriglyceridemia by Dr. Fielding. The studies described were designed to determine the role of cells in lipoprotein metabolism and were performed by incubation of either cultured vascular smooth muscle cells or fibroblasts with diluted freshly drawn plasma. The studies demonstrated that the cells contribute free cholesterol to the plasma and that this cholesterol is trapped as ester which does not enter the cells. The ester is synthesized as the result of LCAT activity and is transferred as part of a transfer complex to VLDL and LDL, which release free cholesterol into the medium. In hypertriglyceridemia there is an increase in plasma LCAT activity with more of the cholesteryl ester going to HDL which has been formed in increased amounts after feeding. In this situation, the HDL releases free cholesterol to the complex, where it is esterified and redistributed. In the discussion Dr. Fielding described the transfer complex involving LCAT and apo D.

Future Research

Following the individual presentations, a panel consisting of Drs. Howard Eder, Bryan Brewer, and Richard Havel considered the evidence relating plasma triglyceride to cardiovascular disease and noted the areas where additional research is needed. Among the points considered were:

1. There is need to recognize the heterogeneity of triglyceride-rich lipoproteins in terms of their physical properties and chemical composition (especially with respect to apoproteins and their unique metabolism). Clearly, separation by ultracentrifugation is no longer sufficient. Not only does it inadequately separate polydisperse lipoproteins, but it also alters apoprotein composition; new methods for separation must be used or techniques working in whole plasma systems must be explored. Identification of sites of origin (e.g., by differences in apo B or presence of A-apoproteins) has been and will be of value in this respect.

2. Techniques for quantification of apoproteins and their subclasses, such as the apo B forms, and apo E and apo A-1 isoforms, require development.

3. The recognition of various lipoproteins by cell surface receptors requires further study, both with respect to the nature of the specificity and other determinants of specificity, such as the lipid composition of the lipoproteins.

It was the conclusion of the panel that metabolic data indicate a relationship between triglyceride-rich lipoproteins and atherosclerosis. Relevant to this were the studies showing chylomicron
remnant uptake by arterial tissue; the conversion of triglyceride-rich lipoproteins into particles whose atherogenicity is well established, such as LDL; and the contribution of components of triglyceride-rich particles to HDL. Study of these problems is important to an understanding of the role of triglyceride-rich lipoproteins in atherosclerosis.

The panel concluded by emphasizing these associations between triglyceride-rich lipoproteins and atherosclerosis. They also emphasized that current knowledge is not adequate to justify its application on a large scale to clinical or epidemiologic studies. However, it was agreed that the biologic data require the conclusion that triglyceride-rich lipoproteins may play a role in atherosclerosis.

Familial Hyperlipoproteinemia and Hypertriglyceridemia

Dr. Brunzell described genetic and metabolic aspects of familial hypertriglyceridemia (FHTG) and familial combined hyperlipoproteinemia (FCHL). He stated that the risk of myocardial infarction was greater in familial combined hyperlipoproteinemia than in familial hypertriglyceridemia and that risk in the latter group may or may not exceed that of “normals,” depending on patient source. In FHTG, there is an increased triglyceride:cholesterol ratio, higher VLDL triglyceride, and larger particle diameter than in FCHL; lipoprotein lipase is normal; the triglyceride secretion rate is about twice that in FCHL; and LDL cholesterol is independent of VLDL triglyceride levels. In FHTG, there may be an increased rate of cholic acid synthesis; a similar increase has not been observed in patients with FCHL. In FCHL, an inverse relationship exists between LDL cholesterol and VLDL triglycerides and this also holds true for LDL apo B and VLDL apo B. In FHTG, the apo A-1/apo A-2 in HDL is normal, while in FCHL the ratio is decreased, owing to less apo A-1 and more apo A-2. Available data on FCHL suggests a monogenic disease with increased levels of apo B. The increased incidence of myocardial infarction in FCHL could be related to increased levels of LDL-2, smaller VLDL, or an abnormal apo A-1/apo A-2 ratio; it is less likely to be related to triglyceridemia, per se. A primary difference between FHTG and FCHL is believed to be hyper apo B in the latter.

Type III Hyperlipoproteinemia

Dr. William Hazzard discussed aspects of Type III hyperlipoproteinemia (HLP), particularly metabolic differences between triglyceride-rich normal α-2 VLDL and cholesterol-rich beta-VLDL. The α-2 VLDL gives rise to higher levels of IDL and LDL, whereas beta-VLDL apparently disappears without going through this normal catabolic pathway. Thus, it appears that beta-VLDL, which is lacking apo E, has a direct removal pathway. Information on apo E phenotypes was presented, including the finding that of 422 patients with MI, five had the dd phenotype and Type III HLP.

Dr. Richard Gregg summarized metabolic studies in Type III HLP: apo E (mol.wt., 34,000) is found in all lipoproteins, predominantly in VLDL and HDL; apo E may inhibit LPL; animals with induced hypercholesterolemia have elevated apo E and develop atherosclerosis; and defects in Type III phenotypes include the presence of an abnormal apo E, the absence of apo E, and apo E receptor defects.

To study apo E metabolism, iodinated apo E was mixed with normal plasma and its distribution was shown to be largely between VLDL and HDL. Injection of this plasma into normal (apo E3+) subjects showed that greater than 90% was cleared in 1 day. Similar clearance curves were obtained when iodinated VLDL was injected, suggesting comparable metabolism of apo E presented by either method. Apo E3+ obtained from normal or Type V subjects and apo E2− obtained from Type III subjects were injected into normal and Type III HLP subjects; in each group the E2− was catabolized more slowly than the E3+. From this information, the following conclusions were made: apo E levels in Type III patients are elevated probably because of a decreased catabolic rate; apo E in Type III migrates abnormally; apo E shows abnormal lipoprotein binding pattern and residence time due to decreased FCR.
**VLDL Effects on Cholesterol Esterification**

Dr. Bersot described studies on effects of various VLDL (from E3 homozygotes) on cholesterol esterification by peritoneal macrophages. These VLDL all demonstrated increased cholesterol esterification (suggesting increased endocytosis) in a manner comparable to the effects of beta-VLDL. In discussion, Dr. Havel presented evidence that one of his Type III patients has a predominance of apo E3 isoform and that uptake by the liver appears normal, suggesting of an apo E receptor defect.

**Monodisperse and Polydisperse LDL**

Dr. Waldo Fisher described extensive studies on monodisperse and polydisperse LDL in relation to hypertriglyceridemia and CHD. With respect to the molecular weight of monodisperse LDL (major mass within a narrow density spread), he made the following observations: there was no change with age, no statistical difference between males and females, no difference in clinically overt atherosclerosis, no difference in hypercholesterolemia; there was a direct genetic correlation (parents vs. siblings). The major molecular weight component is 2.5 X 10^6 as compared with 3.1 X 10^6 in monodisperse LDL.

With respect to polydisperse LDL, the following observations were made: it is correlated to hypertriglyceridemia and the extent of polydispersity is modified by reducing triglyceridemia; it is associated with atherosclerosis when hypertriglyceridemia or diabetes is present but to a much lesser extent in the absence of diabetes.

During the discussion, it was mentioned that similar results are obtained when the d = 1.019–1.063 g/ml fraction is studied. Nevertheless, Dr. Fisher considered that IDL represents part of polydisperse LDL. It was also pointed out that high carbohydrate or fat meals resulted in a shift in polydisperse LDL to larger particles. It was suggested by Dr. Ginsberg that there may be metabolic differences in different LDL. LDL with increased triglyceride content (such as in hypertriglyceridemia) have an increased fractional catabolic rate, and this is shifted downward toward normal during weight reduction.

**Estrogens, Alcohol, and Diabetes**

Dr. Nikkila discussed the effects of estrogens, alcohol, and diabetes on hypertriglyceridemias, concentrating largely on estrogen effects in pre- and postmenopausal women. In women using oral contraceptives, triglyceride levels were found to be higher than controls at all ages up to 45. However, different preparations give different responses (Ethinylestradiol generally causes an increase in plasma triglycerides, whereas Levo-norestril, which also has androgenic activity, does not generally influence triglyceride levels). Neither alone had any effect on HDL, but combined there was a variable decrease in HDL; when evident, the decrease was in HDL-2 with no change in HDL-3.

In postmenopausal women, estradiol had no effect on total triglyceride or in VLDL or LDL triglyceride but appeared to increase triglycerides in HDL. In hypertriglyceridemias, estrogen treatment over 12 months resulted in a variable decrease in triglycerides, a decrease in LDL cholesterol and an increase in HDL cholesterol.

The overall effects of estrogens were summarized as follows: synthetic or conjugated estrogens stimulate the secretion of VLDL, resulting in an increase in plasma VLDL and HDL; natural estrogens have little influence on VLDL secretion, result in a decrease in elevated VLDL levels, and cause an increase in HDL, reflected in HDL-2 levels; and in case control studies, the risk for coronary heart disease (CHD) with oral contraceptives appears to be increased significantly, whereas with natural estrogens, risk is either unchanged or decreased.

The effects of alcohol consumption were summarized as follows: acute intake of alcohol results in increased synthesis and secretion of VLDL, enhanced postprandial chylomicronemia, and increased levels of VLDL and plasma triglycerides; continued use leads to increased LPL activity, increased removal rates of VLDL, increased levels of HDL (HDL-2 and HDL-3), and either continued elevation or decreases in VLDL levels; in subjects with no liver disease, chronic alcohol intake causes variable increases in VLDL and triglycerides, low-to-normal LDL, and elevated HDL levels; in 14 studies on alcohol and CHD, eight report a decreased incidence of CHD, four report an increase and two report no change.

In insulin-treated juvenile diabetics, plasma lipids including VLDL levels were normal. Patients with poor diabetic control exhibited increased VLDL triglycerides, increased serum cholesterol, and decreased HDL levels. In comparing conventional insulin therapy with insulin infusion by pump, it was reported that the latter technique resulted in a reduced insulin requirement and dose, reduced blood glucose levels and lower levels of plasma triglycerides and HDL cholesterol. Finally, in a comparison between HDL cholesterol levels and plasma insulin responses in obese, nondiabetic subjects, it was found that the levels of plasma HDL cholesterol varied inversely with the 2-hour levels of plasma insulin.
Dr. Bierman stated that HDL cholesterol in insulin-dependent diabetics was higher than in controls and that increased insulin doses resulted in decreased HDL cholesterol. In contrast, non-insulin dependent diabetics displayed decreased HDL cholesterol; however, apo A levels were not lower, suggesting that triglyceride had substituted for cholesterol in the HDL. Further discussion by Drs. Antonio Gotto and Gustav Schonfeld centered around the increased levels of glycosylated lipoproteins in diabetes and changes in glycosyl LDL receptor activity.

Fatty Acids

Dr. William Connor presented data on effects of ω-3 fatty acids, commonly found in marine oils, in normal and hypertriglyceridemic subjects. He first reviewed sources of the different polyunsaturated acids, the historical aspects of effects of these acids, the studies in Eskimos who consume large quantities of ω-3 fatty acids, and the differences between ω-3 and ω-6 fatty acids in the prostaglandin (PG) synthetic pathways (e.g., platelets versus endothelial PG synthesis). A recent significant finding in this latter area is that the ω-3 fatty acids produce functional PG (PGI₂ or prostacyclin) in endothelial cells, but produce less functional thromboxanes (TX₃) in platelets, ultimately reducing platelet aggregability and interaction with the arterial intima.

The following findings were reported: administration of salmon oil to control subjects resulted in a decrease in plasma lipids and triglycerides. This was a reflection of decreased VLDL and LDL cholesterol, whereas HDL cholesterol remained unchanged. Safflower oil, in contrast, resulted in a decreased plasma cholesterol but no change in plasma triglycerides; administration of salmon oil to seven Type II B patients resulted in a decrease in cholesterol from 368 to 270 mg/dl, and in triglycerides from 345 to 115 mg/dl. In seven Type V patients, cholesterol levels were reduced from 1000 to 303 mg/dl on low fat diets and further reduced to 150 mg/dl when salmon oil was incorporated in the diet. In these same patients, plasma triglycerides were reduced from 1252 mg/dl to final levels of 326 mg/dl by salmon oil ingestion. In subjects on salmon oil, bleeding times were significantly increased and platelet retention on glass beads was significantly decreased. In addition, platelets were larger than normal and had an eicosapentenoate:arachidonate (μg%) ratio of 0.3:1.0 as compared with 0.0045:1.0 in controls.

Coronary Heart Disease

Dr. George Rhoads discussed triglycerides and postmortem lesions in CHD. He first discussed aspects of univariate and multivariate analysis and bias of sample acquisition. From univariate analysis, he indicated the relative risk of nonfasting triglyceride levels and CHD to be about 2. For total mortality, however, there was no relationship.

For the multivariate analysis, adjustment for age did not eliminate the correlation of plasma (nonfasting) triglycerides and CHD. However, when adjustment was made for systolic blood pressure, obesity, cigarette smoking, alcohol, and plasma glucose levels, the significance of plasma triglycerides and CHD risk was lost. He reported no relationship of fasting triglyceride levels and atherosclerosis from autopsy studies. Furthermore, systolic blood pressure, cholesterol, and cigarette smoking were related to aortic atherosclerosis but not to the coronary disease.

Dr. Alick Little presented studies on a patient with apo C-2 deficiency, who died at age 63 showing no evidence of atherosclerosis. No fat infiltration was seen in either the liver or spleen and only a few foam cells were evident in bone marrow. He and his homozygous relatives all displayed marked chylomicronemia and plasma triglycerides of over 10,000 mg/dl, most of which was in chylomicrons.

Dr. Gotto chaired a panel on clinical research which included Drs. Virgil Brown, Esko Nikkila and Edwin Bierman. The following questions were addressed:

- How strongly does the clinical evidence relate triglycerides to CHD?
- What additional clinical research is needed to determine whether triglycerides are a risk in CHD?

In general, this panel conceded the dilemma regarding the relationship of hypertriglyceridemia and CHD, but also emphasized that there was little doubt that certain subsets of the population with hypertriglyceridemia displayed predisposition to CHD, and that this relationship was not uncovered in population studies. The following points and recommendations were made:

1. Subsets of the population need to be defined and emphasis should not be limited to determination of fasting triglyceride levels. Genetic markers for the monogenic disorders need to be defined (e.g., E₃ deficiency, population frequencies of FCHL and FHTG).

2. The distribution of triglycerides in the various lipoproteins requires consideration, and in particular simultaneous data on HDL and TG production rates are needed. Problems posed during this discussion included:
a) the reason for low HDL levels in most hypertriglyceridemias, where the disorder appears primarily as overproduction of some component of VLDL; and b) the apparent discrepancy between TG production rates and apo B production rates.

3. There is a need to develop and apply appropriate methods for analysis of HDL subfractions that are appropriate for use in clinical and epidemiologic studies.

4. In general, it was agreed that future prevalence and intervention trials require a better data base.

Summary

Dr. Robert Levy concluded the Workshop by summarizing the advantages and limitations of each of the major approaches addressed by the Workshop — the epidemiological, basic research and clinical research studies. He also discussed the limitations of our current knowledge from both the research and public health aspects relating hypertriglyceridemia and CHD. He concluded that, although the evidence suggesting hypertriglyceridemia as an independent risk factor in CHD was still in some doubt, hypertriglyceridemia is a marker for other disorders directly related to CHD. Dr. Lippel gave brief closing remarks.

Acknowledgments

The authors thank Janet Bungay for editorial assistance and Doris McDonough for manuscript preparation.
Relationship of hypertriglyceridemia to atherosclerosis.
K Lippel, H Tyroler, H Eder, A Gotto, Jr and G Vahouny

doi: 10.1161/01.ATV.1.6.406

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/1/6/406.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/