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Aspen Hepatic Cholesterol and Lipoprotein Conference 1989. Report of a Conference

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The Aspen Cholesterol Conference was held in Aspen, Colorado August 19th to 22nd, 1989. The theme of this conference was the role of diet in the regulation of lipoprotein metabolism. This conference is designed primarily to provide young investigators with the opportunity to hear an up-to-date review of current research in the field of cholesterol metabolism, with emphasis on the role of the liver and gut in the regulation of lipid metabolism. The following briefly summarizes the presentations at this conference.

Dr. Donald Small opened the meeting by discussing the physical chemistry of lipoprotein surfaces. The surfaces of triglyceride rich lipoproteins are made up of a continuous phase of phospholipids in which other lipids and apolipoproteins are embedded. The other lipids include cholesterol, small amounts of core components (triglycerides and cholesteryl esters) and, during lipolysis, fatty acids and partial glycerides. Dietary fatty acids are incorporated into the phospholipids of nascent chylomicrons. The composition of phospholipid fatty acids changes with different dietary fats, and therefore there are potential changes in the physical properties of the surface that vary between loosely packed and nearly solid surfaces. The cholesterol content of nascent chylomicrons is very low, but increases when these particles enter plasma and increases even further with lipolysis of triglyceride. As the unesterified cholesterol-to-phospholipid ratio approaches one, the triglyceride content of the surface falls from about 2.5 mol % (relative to phospholipid) to nearly 1.0 mol %, thus removing the substrate for lipase from the surface.

Triolein emulsions can be used as models for chylomicrons and their remnants. Emulsions with fluid surfaces and low unesterified cholesterol content bind primarily apoproteins C, are rapidly lipolyzed, and are also rapidly removed by the liver from plasma of rats; trivial amounts enter the spleen. These particles model normal chylomicron metabolism in rats fed triolein. Egg yolk phosphatidylcholine with high cholesterol content forms a viscous surface, binds only 1/10 as much protein as the low cholesterol emulsions, and the major protein is albumin, although small amounts of other apolipoproteins, including apoE, will bind. These emulsions are not hydrolyzed but are very rapidly removed from plasma with a liver/spleen uptake ratio of 6.6. When viscous surfaces are produced by making emulsions with saturated lecithin and high cholesterol content, then lipolysis is blocked and removal from plasma is very slow and uptake is directed to the spleen. When the emulsion surface is at the gel-liquid crystal transition (dipalmitoyl phosphatidylcholine-low cholesterol), more protein is adsorbed, with the major banding moiety a 55-58 kDa protein. These emulsions are not hydrolyzed and are removed slowly with the liver/spleen ratio of 5.3. Finally, when the surface of the emulsion is largely solid (distearoyl phosphatidylcholine-low cholesterol), then little protein adsorbs, no hydrolysis occurs, but removal from plasma is fast with the liver/spleen ratio of 8.6. In liver perfusion studies, emulsions lacking adsorbed proteins are not taken up. Thus, lipid surface composition and physical properties govern the amount and type of protein adsorbed from plasma and this, in turn, dictates metabolism and tissue distribution.

Dr. Francis Simon considered the polarized organization of liver surface membrane lipids and some of its functional correlates. The surface membrane of epithelial cells, including the liver, is organized into two domains, apical (or bile canalicular) and basolateral (or sinusoidal). The surface membrane domains differ in protein and lipid composition as well as in their fluidity. Dr. Simon focused on three areas: *1*) lipid composition and fluidity of liver surface membrane subfractions; *2*) importance of lipid structure on the activity on the activity of NaK-ATPase; and *3*) involvement of sinusoidal membrane lipids in the pathogenesis of cholestasis.

Liver plasma membrane subfractions can be isolated simultaneously in good yield and purity. The two major membrane domains have similar total fatty acid composition, but apical membranes have a higher content of cholesterol and sphingomyelin than the basolateral membrane which is more fluid. In biological membranes, fluidity consists of two major components: structural and dynamic. Using time-resolved fluorescence polarization, the

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structural parameter is found to be more rigid in the apical surface, but the dynamic component demonstrates a faster rate of lateral mobility than the sinusoidal surface. Since previous studies have demonstrated NaK-ATPase enzyme molecules but no enzyme activity on the apical surface, the possibility that the rigid apical membrane inhibits expression of NaK-ATPase activity was examined. In vitro addition of agents known to fluidize the apical membrane results in activation of latent enzyme activity selectively on the apical surface. This phenomenon was only demonstrated in liver. The biochemical basis of the dual localization of the enzyme is unclear but may be related to different NaK-ATPase isoenzymes in each membrane domain or, alternatively, to the apparent absence of the beta subunit from the apical membrane.

In other studies administration of the synthetic estrogen, ethinyl estradiol, causes decreased bile flow and hepatic bile salt transport. Estrogen administration decreases sinusoidal NaK-ATPase activity and Na-dependent bile salt uptake. These changes accompany decreased sinusoidal fluidity without changes in apical or microsomal membranes. In summary, intracellular mechanisms sort lipids to different surface domains and this has functional correlates. Drugs and nutrition may selectively change membrane function through specific alterations in membrane composition and fluidity.

Dr. Arthur Spector discussed the effect of dietary lipids on hepatic membrane composition and function. Tissue culture studies have demonstrated that membrane fatty acid composition can be changed substantially in intact cells by adding different fatty acid supplements to the growth medium. The question under consideration was whether similar fatty acid compositional changes can also occur in tissue membranes when animals are fed diets containing different fats, and if so, would this produce changes in membrane function. To examine this question, rats were fed semi-purified diets containing 14% saturated fat (coconut oil) or an n-6 polyunsaturated fat (sunflower seed oil). Liver microsomes obtained from rats fed sunflower seed oil contain substantially more polyunsaturated fatty acid, especially arachidonic acid, in membrane phospholipids than those obtained from coconut oil-fed rats. Differences were apparent within 3 days and were maintained for 70 days of feeding. There was no difference, however, in the phospholipid or cholesterol content of the two sets of microsomes.

Acyl coenzyme A:cholesterol acyltransferase (ACAT) was measured in isolated liver microsomes after addition of radioactive palmitoyl coenzyme A but no exogenous cholesterol. Similar amounts of cholesterol were inherent in both sets of microsomes. ACAT activity was 1.5- to 3-times higher in the microsomes from sunflower seed oil-fed animals than from the coconut oil-fed animals. Higher ACAT activities occurred throughout the 70-day feeding period. The transition temperature (by Arrhenius plot) of ACAT activity in the two microsomes was the same, as was their activation energy and palmitoyl-CoA hydrolase activity. From these results it seems unlikely that the change in ACAT activity is due to a microenvironmental lipid effect on the enzyme or any difference in the access of palmitoyl-CoA. Higher ACAT activity occurred in microsomes isolated from the livers of rats fed the highly saturated cocoa butter as compared with microsomes from rats fed menhaden oil, which is rich in n-3 polyunsaturates.

An effect of membrane fatty acids on ACAT activity was also obtained by incubating hepatic microsomes isolated from rats fed ordinary laboratory chow with phospholipid exchange protein and liposomes containing different phosphatidylcholines. After an initial 2-h incubation, microsomes incubated with dioleoyl phosphatidylcholine exhibited 1.5-times higher ACAT activity than microsomes incubated wth dipalmitoyl phosphatidylcholine. Microsomes incubated with dioleoyl phosphatidylcholine were enriched in unsaturated fatty acids, whereas those incubated with dipalmitoyl phosphatidylcholine were enriched in saturated fatty acids. It was not possible to test the effect of polyunsaturated fatty acids in this system because microsomal ACAT activity was rapidly inactivated by incubation with dilinoleoyl phosphatidylcholine liposomes.

Lipid peroxidation also was higher in microsomes from sunflower seed oil-fed rats than from those fed coconut oil. Peroxidation was measured through malondialdehyde formation after addition of NADPH, $FeCl_3$, and ADP. Peroxidation increased progressively as the polyunsaturated fatty acid content of the microsomes was raised and reached maximal levels within 10 days. The microsomal phospholipid fatty acid that was consumed to the greatest extent in the peroxidation was arachidonic acid. Furthermore, enrichment with arachidonic acid by incubation of microsomes with diarachidonoyl phosphatidylcholine and phospholipid exchange raised peroxidation almost to the level obtained with microsomes from animals fed sunflower seed oil.

These findings indicate that increases in dietary polyunsaturated fatty acids can rapidly influence the fatty acid composition and function of liver membranes. The effects of ACAT probably are due to structural changes within the lipid bilayer that increases either the access of cholesterol to ACAT or the ability of the product, cholesteryl ester, to diffuse away from the enzyme. By contrast, the effect on peroxidation appears to be due to an increase in the microsomal arachidonic acid, which provides more substrate for peroxidation. Based on these results, the possibility exists that large intakes of polyunsaturates may alter the normal processing of cholesterol in the liver and also make the microsomal membranes more sensitive to peroxidative injury.

Dr. Thomas Brasitus discussed the effects of dietary

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modification of hepatic and intestinal cell membranes on their activity. It has become increasingly clear that various dietary manipulations can influence the lipid composition, fluidity, and certain protein-mediated activities of the antipodal plasma membranes of rat enterocytes and hepatocytes. Three specific examples were discussed. Alterations in the saturation of dietary triacylglycerols can influence the lipid composition, fluidity, and several enzymatic activities of small intestinal brush border and basolateral membranes of rats. With the feeding of 37% corn oil over a 3-week span, there is no change in brush border membrane fluidity, a phenomenon that is attributable to compensatory adaptation of the cholesterol/phospholipid ratio. This adaptation affects brush border only. Also, increase in intraluminal calcium, effected by intragastric gavage, altered lipid composition, fluidity, and activity exclusively in proximal intestinal brush border membranes. Moreover, these in vivo effects can, in general, be reproduced by in vitro incubation of membranes with calcium. Of interest, the latter in vitro effects of calcium also have been shown to occur in rat hepatic sinusoidal membranes but not in canalicular membranes. Calcium infusion increases the amount of sphingomyelin by increasing sphingomyelin synthesis and decreasing its degradation. Dr. Brasitus has shown that the estrogen-induced effects on lipid composition and fluidity of rat hepatic plasma membranes, as well as on hepatic bile flow, can, at least in part, be restored to control levels by a "starve-re-fed" dietary regimen.

Dr. Richard Havel reviewed receptor and nonreceptor uptake of lipoproteins and their intracellular fates. The high affinity of ^{45}Ca binding to the low density lipoprotein receptor and the LDL-receptor-related protein (LRP) was utilized to study the subcellular distribution of these two proteins in rat liver. Like LDL-receptor, LRP was enriched many-fold in rat liver endosomal membranes with a relative distribution in early and late endosomal compartments consistent with recycling between endosomes and the cell surface. The high concentration of LRP in hepatic endosomal membranes greatly facilitated demonstration of Ca-dependent binding of apolipoprotein (apo) E- and apoB-containing lipoproteins in ligand blots. LRP was several-fold more abundant than the LDL-receptor in hepatic parenchymal cells, and showed an extensive degradation in hepatic endosomes; LRP was also found in high concentrations in the Golgi apparatus and endoplasmic reticulum. These data suggest a high rate of synthesis of LRP that appeared to be unaffected by treatment of rats with estradiol. The repeating cysteine-rich A-motif found in the ligand-binding domain of LRP appeared to be responsible for Ca binding by LRP, LDL-receptor, and complement factor C9, and accounted for immunological cross-reactivity among these proteins. Weaker ligand-blotting properties and an extraordinary susceptibility to proteolysis most likely contribute to the difficulty of detecting LRP in conventional assays for lipoprotein receptors. Results suggest a unique endosomal proteolytic processing of this protein and are consistent with a functional role of LRP in lipoprotein metabolism.

Dr. Helen Hobbs examined the status of molecular definition of mutations at the LDL-receptor locus that cause familial hypercholesterolemia (FH). Over 34 different mutations have been characterized at a molecular level in the laboratory of Drs. Michael Brown and Joseph Goldstein, and these mutations include insertions, deletions, nonsense and missense mutations. Almost every unrelated individual with FH so far tested has had a different mutation with four exceptions. In the French Canadian population, five different mutations, two deletions and three missense mutations, comprise 76% of the mutant LDL receptor alleles in 130 FH heterozygotes from the Montreal area. Twelve FH homozygotes of Afrikaner descent were analyzed and over 95% of the alleles had one of two different missense mutations. Previous studies have demonstrated that there is a nonsense mutation in exon 14 of the LDL receptor gene that is found commonly and exclusively in the Christian Lebanese community. In addition, a large deletion at the 5' end of the LDL receptor gene has recently been cloned in another laboratory, and this mutation occurs in about 50% of FH of patients in the Finnish population. In each of these four populations, the high frequency of a small number of mutant LDL receptor alleles causing FH is most likely due to the founder effect. Direct detection of LDL receptor mutations in these populations is feasible for prenatal diagnosis or to confirm the clinical diagonsis of FH. In most populations, however, the molecular diagnosis of FH will require the use of genetic markers, i.e., restriction fragment length polymorphisms (RFLPs), in the context of a pedigree analysis.

Ten RFLPs at the receptor locus were used to construct LDL receptor haplotypes in 123 Caucasian American individuals, and 31 different LDL receptor haplotypes were identified. Using all ten RFLPs, the heterozygosity index is 85%. These ten RFLPs were used to analyze an unusual pedigree with FH. In this pedigree, there are multiple individuals who have a mutation in the LDL receptor gene and have a normal LDL-cholesterol level. Among the FH heterozygotes in this family, there is a bimodal distribution of LDL-cholesterol levels that is consistent with a single gene causing a lowering of the LDL-cholesterol level. The candidate gene approach was used in the attempt to identify the gene responsible for the LDL-lowering effect in this family. Segregation analysis was performed using genetic markers associated with LDL receptor gene, the apoB gene, and the closely linked apoE-CI-CII genes. For each locus, the four parental alleles could be differentiated and no allele co-segregated with the LDL-lowering effect. Other candidates genes including apolipoprotein C-III, hepatic lipase, HMG-CoA reduc-

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tase, and the LDL receptor-related protein are presently being evaluated to determine whether a particular allele co-segregates with the LDL-lowering effect in this family.

Dr. Karl Weisgraber discussed factors that regulate hepatic uptake of apoE-containing lipoproteins. That apoE plays an important role in hepatic uptake of plasma lipoproteins has been clearly established. ApoE mediates uptake of chylomicron remnants, VLDL, IDL, and HDL- with apoE. Three factors that influence uptake were considered: receptor-binding activity, lipoprotein distribution, and the quantities of apoE available in plasma for uptake. Under receptor-binding activity, the association of variants of apoE with defective receptor-binding activity with type III hyperlipoproteinemia was considered. The focus was on dominant versus recessive expression of the disorder. Recessive expression is more common and is associated with apoE2 ($Arg_{158}\rightarrow Cys$), the most common variant of E2. The apoE3 ($Cys_{112}\rightarrow Arg$, $Arg_{142}\rightarrow Cys$) variant was used as an example of dominant expression. A distinguishing feature between the two variants is that the 158-cysteine variant's binding activity can be modulated (from high affinity to low affinity) by lipid environment or chemical modification with cysteamine, whereas the 142-cysteine variant's activity cannot be modulated and its binding activity is always defective. It was suggested that the difference between the two variants is that the 158 site does not interact directly with the LDL receptor but helps to maintain the receptor binding region of apoE in a proper binding conformation. Arginine or the lysine analogue generated by cysteamine modification can serve this purpose. In contrast, the 142 site occurs in a putative α-helix enriched in basic amino acids that is thought to interact directly with the receptor. The lysine analogue does not substitute for arginine in this case, and thus modification of this site (the specific requirement) does not result in modulation of activity.

Under lipoprotein distribution, it was demonstrated that positive charge at position 112 accounted for the preference of apoE4 for VLDL. The preference of apoE3 for HDL was the result of an inherent property of the apoE3 monomer and the formation of apoE3-A-II copolymers. Results from apoE infusion studies in cholesterol-fed rabbits and from studies in which apoE was added to lymph suggested the quantities of apoE available in plasma may be rate-limiting for the processing and/or uptake of lipoproteins.

Prof. Gerd Utermann's topic was genetics of the atherogenic Lp[a] lipoprotein. The Lp[a] lipoprotein is a macromolecular complex in human plasma that is assembled from an LDL and the Lp[a] glycoprotein (= apo[a]). Apo[a] exhibits a high degree of homology to the plasma zymogen plasminogen. Thus the Lp[a] lipoprotein combines structural elements of the lipoprotein and the blood clotting systems. Lp[a] lipoprotein levels vary 1000-fold between subjects and represent a continuous quantitative genetic trait with a highly skewed distribution of levels in Caucasian populations.

High levels of Lp[a] are associated with an increased risk for premature coronary heart disease. The genetics of the Lp[a] trait remained mysterious until the recent discovery of genetically controlled size polymorphism of apo[a] that is determined by a series of at least seven alleles (designated Lp^F, Lp^B, Lp^{s1}, Lp^{s2}, Lp^{s3}, Lp^{s4}, Lp^0) at the apo[a] structural gene locus which codes for protein species from about 400 kDa to 700 kDa. This size heterogeneity is caused by a variable number of kringle four repeats in the apo[a] gene. The intron–exon structure in the kringle 4 repeat region of the gene is highly conserved suggesting a very recent evolutionary origin. The apo[a] gene was localized on chromosome 6q2.6–q2.7 and identified as the long-sought major gene for Lp[a] plasma concentrations; it explains more than 40% of the total variability of Lp[a] levels in Caucasians. Apo[a] alleles may interact with genes at other loci, e.g., mutant LDL-receptor alleles, in a multiplicative way resulting in extremely high Lp[a] levels in some patients with familial hypercholesterolemia. Such patients have a significantly increased risk for early coronary heart disease.

Dr. John Dietschy discussed the effects of changes in dietary cholesterol and triacylglycerol content on LDL-cholesterol metabolism in hamsters. Male hamsters were chosen for study since they respond to dietary manipulation in a manner similar to man. The following four parameters were determined: the LDL-cholesterol production rate (J_d), the maximum achievable rate of LDL transport by the LDL receptor (J^m), the functional affinity of the LDL particle for the LDL receptor (K_m), and the rate of LDL transport independent of the LDL receptor (P). An increase of cholesterol in the diet resulted in a dose-dependent increase in steady-state LDL-cholesterol concentration. The rise in LDL-cholesterol was due to a decrease in receptor-dependent LDL transport and a small increase in LDL-cholesterol production. The cholesteryl ester content of the liver rose and the hepatic synthesis of cholesterol was suppressed in the animals fed a high-cholesterol diet.

When triacylglycerols were added to a diet rich in cholesterol, the increase in LDL-cholesterol production rate was much more pronounced than that seen in the animals fed cholesterol alone. The increase in production was proportional to the amount of triacylglycerol given. Triacylglycerols with different types of fatty acids had a dramatically different effect upon receptor-dependent LDL transport. Saturated fatty acids augmented the suppressive effect of dietary cholesterol on receptor-dependent LDL transport (J^m) and this resulted in a marked increase in plasma LDL-cholesterol concentrations. The effect of unsaturated fatty acid was to increase J_d as well as J^m so that there was relatively little change in the plasma LDL concentration. The changes induced in LDL

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receptor activity by triacylglycerols were independent of either the rate of hepatic cholesterol synthesis or the hepatic cholesterol ester content.

Dr. Andrew Kandutsch discussed the role of intracellular oxysterol metabolites in the regulation of cholesterol metabolism. In cell culture studies, HMG-CoA reductase is repressed by certain oxysterols, and it has been postulated that the action of oxysterols is mediated by a protein that binds oxysterols but not cholesterol. He identified a protein that bound oxysterols, and a monomer of that protein that has an apparent molecular weight of 97 kDa was isolated. A polymeric form of the monomer seemed to be converted into a dimer after binding to an oxysterol. Dr. Kandutsch postulated that regulatory oxysterols are generated intracellularly in concentrations sufficient to regulate reductase activity. In cell-culture studies using Chinese hamster lung cells he found two oxysterols with regulatory activity: 24(S) 25-epoxycholesterol and 25-hydroxycholesterol. When mevalonate was added, two additional oxysterols, 32-hydroxylanosterol and 32-oxolanosterol, appeared at the same time that the level of reductase declined.

Dietary cholesterol has been shown to repress hepatic HMG-CoA reductase in vivo, though the mechanism by which this effect is mediated has not been elucidated. When mice are fed a high-cholesterol diet, concentrations of 24(S)-, 25-, and (25R)-26-hydroxycholesterol increased in the liver coincident with a reduction in the HMG-CoA reductase activity. The stereochemistery of the 24- and 26- substitutions indicate enzymatic hydroxylation of cholesterol, and concentrations of 24-hydroxycholesterol increased more than any of the other oxysterols. This sterol had not previously been found in liver but had been isolated from brain and adrenal tissue. It is not an intermediate in any known metabolic pathway and its potential role as a regulator of sterol synthesis is undergoing continued investigation.

Dr. Paul Dawson discussed the purification of cDNA cloning of the oxysterol-binding protein. As had been reviewed by Dr. Kandutsch, feedback repression of several enzymes of the cholesterol biosynthetic pathway and the LDL receptor is mediated by oxysterols. To examine the role of the oxysterol-binding protein in this regulation, the protein was purified more than 40,000-fold from liver using a series of ion-exchange and gel filtration chromatography steps. The final preparation of oxysterol-binding protein contained a doublet of peptides with molecular weights 1010 and 96 kDa. The components oligomerized and migrated on gel filtration with an apparent molecular weight of 280,000 in the absence or presence of oxysterols. Using oligonucleotides corresponding to sequences from the purified protein, a rabbit liver cDNA was cloned. The predicted amino acid sequence revealed a protein of 809 amino acids with: *a*) a glycine- and alanine-rich region (63% of 80 residues) at the amino terminus, and *b*) a 35-residue leucine zipper motif that may mediate the previously observed oligomerization of the protein. After transfection into simian COS cells, the rabbit cDNA produced a protein with the same affinity and specificity for sterols as the previously purified liver protein. Immunoblotting analysis showed that the rabbit cDNA encodes both the 97 to 101 kDa forms of the oxysterol-binding protein. The role of the oxysterol binding protein's leucine zipper in oligomerization was examined by glutaraldehyde cross-linking of truncation mutants expressed in COS cells. This analysis indicated that the oxysterol-binding protein is a dimer under native conditions and that a domain including the leucine zipper is required for the dimerization. The availability of the cDNA and its ability to be expressed in animal cells will now allow further examination of the oxysterol binding protein's role in oxysterol and cholesterol metabolism.

Dr. Godfrey Getz discussed recent work that has implicated the regulation of the activity and expression of the hepatic LDL receptor in accounting for the differential effects of saturated and unsaturated fat on plasma lipoprotein levels. His presentation reported on dietary experiments in the baboon, an animal with the HDL-cholesterol/LDL-cholesterol ratio of 2.5, in contrast to the ratio in human plasma of 0.4. In baboons fed cholesterol and coconut oil, the hepatic LDL receptor mRNA was more significantly down-regulated than when cholesterol was fed with olive oil as the major fat. In coconut oil-fed animals, LDL receptor message was negatively correlated with HDL-cholesterol and apoprotein A-I levels. A negative correlation of hepatic message and HDL-cholesterol was also seen in a more complex experiment using carefully phenotyped animals in which lard and corn oil were the major dietary fats. In this experiment a repeated measures design was used, so that each animal served as its own control. However, there was also a strong negative correlation with LDL-cholesterol in the plasma.

In an attempt to understand the potential regulatory properties of baboon lipoproteins, these were incubated with HepG2 cells, as indicator cells for 4 or 24 h. Baboon LDL, whether from animals fed coconut oil or olive oil, down-regulated hepatic LDL receptor mRNA to the same extent. Baboon HDL, when incubated for 24 h, behaved as a cholesterol donor to HepG2 cells, but HDL from coconut oil-fed animals was apparently a more effective cholesterol donor than HDL from olive oil-fed animals. When incubation was only for 4 h, HDL behaved as a cholesterol acceptor, up-regulating hepatic LDL receptor and HMG-CoA synthase mRNA, again with differences between HDL from coconut oil- and olive oil-fed animals. The former was a significantly less effective cholesterol acceptor. The differential effects of HDL were a function of HDL_2, not HDL_3. The quantitatively different effects of HDL_2 correlated with differences in their phospholipid fatty acid composition. These data sug-

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gested that in an animal such as the baboon, the regulation of hepatic LDL receptor may depend on the concentration and functional properties of individual HDL subclasses in the plasma, as well as on LDL.

Dr. Jan Breslow indicated that risk for coronary heart disease is inversely proportional to HDL-cholesterol levels. Intense effort is being expended in his laboratory to understand the genetic regulation of HDL-cholesterol levels. HDL particles are formed in plasma by a complex set of events. The building blocks appear to be the apolipoproteins, apoA-I and A-II, phospholipid, and free cholesterol. Sizes and amounts of HDL also are determined by processing proteins such as lipoproteins lipase, hepatic triglyceride lipase, lecithin:cholesterol acyltransferase, and cholesteryl ester transfer protein. Rare inborn errors of metabolism have been described that affect HDL-cholesterol concentrations, but do not explain the variation in HDL concentration in the population. Dr. Breslow noted that turnover studies of HDL apolipoproteins reveal that in subjects on a high-fat diet the HDL-cholesterol concentration correlates best with apoA-I fractional catabolic rate, but when changing from a low-fat to a high-fat diet, the increase in HDL-cholesterol levels correlates best with the change in the apoA-I synthetic rate. Genes controlling apoA-I fractional catabolic rate in a high-fat diet regimen and those controlling apoA-I synthetic rate in response to dietary fat may harbor the loci to explain the common genetic variation in HDL-cholesterol levels in the general population. In other experiments transgenic mice were produced that have the human apoA-I gene and oversynthesize apoA-I. In these mice Breslow and colleagues have shown a direct effect of this gene on HDL-cholesterol concentrations and on dietary responsiveness of HDL-cholesterol levels.

Dr. Jake Lusis' laboratory is interested in the genetic control of the response of cholesterol metabolism to dietary challenge. This is difficult to examine directly in humans, and, therefore, Dr. Lusis is utilizing a mouse model. He stated that three examples of genetic-dietary interactions have been identified and partially characterized among inbred strains of mice. The first controls sterol synthesis and the expression of the enzyme HMG-CoA reductase. Striking genetic differences in the regulation of this enzyme in response to a high-fat diet challenge were observed. Some of these differences appear to result from altered expression of *trans*-acting regulatory factors. Preliminary studies suggest that they do not involve altered expression of the recently identified CNBP protein which binds to the sterol response element.

The second example involves the transcription of the apolipoprotein A-IV (apoA-IV) gene. Dramatic differences in both basal levels and diet-induced changes in apoA-IV gene expression occur among inbred mouse studies. These differences are due at least in part to *cis*-acting elements of the apoA-IV gene on mouse chromosome 9 that controls transcriptional activity. A potential sterol response element that binds the CNBP protein has been identified in the apoA-IV promotor, although it is unclear whether this participates in the dietary regulation of apoA-IV transcription.

Finally, genes have been identified that control susceptibility of mice to diet-induced atherosclerosis. At least one such gene, *Ath-1*, located on mouse chromosome 1, appears to act by altering the catabolism of HDL in response to a high-fat diet, resulting in low HDL levels in the susceptible strains. Tightly linked to the *Ath-1* gene, but apparently distinct from it, is the apoA-II gene. A novel mutation of the apoA-II gene controls apoA-II translational efficiency, resulting in altered levels of plasma apoA-II and dramatic differences in the size of HDL particles.

Robert L. Hamilton reviewed new and published data that apoB synthesis and nascent VLDL-triglyceride synthesis are not tightly coordinated and may occur independently in the rough endoplasmic reticulum (RER) and smooth ER, respectively. The hypothesis was presented that apoB translocation across the RER membrane may be tightly coupled with cholesteryl ester synthesis by ACAT bound to the RER membrane. Thus, ACAT may provide the necessary hydrophobic core molecules for newly synthesized lipophilic apoB for transport in the aqueous milieu of the RER cisternae. Biochemical evidence was presented that French pressure cell treatment of a novel "intact" RER subcellular fraction (characterized electron microscopically as flattened strips of ribosome-studded membranes) released apoB-48. The French pressure cell procedure permits the separation of organelle content proteins from membrane proteins to a large extent. The concept was discussed that a very small primordial complex comprised largely of apoB and cholesteryl esters may be formed in the RER prior to its adsorption to nascent triglyceride-rich particles at the RER-SER junction.

New data were presented on the final stages of nascent VLDL assembly in the Golgi apparatus, based upon an improved "intact" Golgi isolation technique that was shown biochemically to be largely uncontaminated by multivesicular bodies containing remnants. Following release of Golgi content by French pressure cell treatment, virtually all apoB, triglycerides, apoE, proapoA-I, and C apoproteins float at $d < 1.010$ g/ml, whereas albumin and other serum proteins sediment to the centrifuge tube bottom. No particles of size or shape of HDL or LDL were seen in this protein-rich content fraction, as determined by negative staining electron microscopy and almost no free apolipoproteins were found by SDS-PAGE. Therefore, it was concluded that the hepatocyte, under normal dietary conditions, synthesizes a single plasma lipoprotein precursor, namely nascent VLDL, that gives rise to plasma LDL by incompletely understood processes and con-

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tributes to the formation of plasma HDL. This conclusion is consistent with other data and with the chemical of truly nascent VLDL, which were shown to be highly enriched (compared with plasma VLDL) in phospholipids, particularly phosphatidylethanolamine (PE), and have a very low free cholesterol to phospholipid ratio. These data are consistent with those of **Dr. P. E. Fielding**, who has shown that in normal human plasma, a small fraction of VLDL is also enriched in PE to the same extent as rat hepatocytic Golgi VLDL, but differs in that the human "nascent" VLDL lacks apoE (and proapoA-I). Immunogold localization of apoE in rat hepatocytes suggests that apoE (and other soluble apolipoproteins) may be added late in the assembly sequence, perhaps as the nascent VLDL particles accumulate in clusters in the forming secretory vesicles of the Golgi apparatus.

Taken together, these data on intrahepatocytic assembly of nascent VLDL suggest that the metabolism of triglyceride-rich particles in plasma follows a stepwise cascade in which very early events are the dissociation of apoE and proapoA-I together with phosphatidylcholine from the surface of nascent VLDL, representing perhaps one mechanism for the formation of nascent discoidal HDL. Concomitantly, PE also rapidly dissociates from the VLDL particles, possibly in exchange for C apolipoproteins from plasma HDL, thereby facilitating hydrolysis of core triglycerides by capillary lipoprotein lipase. The loss of phospholipids may then permit the surface to adsorb more unesterified cholesterol, perhaps from HDL or even the endothelium. The next step in this hypothetical cascade is transfer of multiple copies of apoE from HDL (to the now free cholesterol-enriched remnant particles), the ligand that facilitates triglyceride-rich remnant uptake by receptor-mediated endocytosis in the space of Disse, initiating remnant catabolism by intrahepatocytic lysosomal enzymes.

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