[Frontiers in Bioscience 6, d332-354, March 1, 2001]

POSTPRANDIAL LIPOPROTEINS AND ATHEROSCLEROSIS

Kenneth C-W. Yu [1] and Allen D. Cooper [2]

[1] Research Institute, Ames Building, Palo Alto Medical Foundation 795 El Camino Real, Palo Alto, CA 94301, and [2] Department of Medicine, Division of Gastroenterology, Stanford University Medical Center, Stanford, CA 94305

TABLE OF CONTENTS

1. ABSTRACT

During the postprandial state, dietary lipid is transported from the intestine to peripheral tissues by plasma lipoproteins called chylomicrons. In the capillary beds of peripheral tissues, chylomicron triglycerides are lipolyzed by the enzyme, lipoprotein lipase, allowing the delivery of free fatty acids to the cells. As a result, this produces a new particle of smaller size and enriched with cholesteryl ester referred to as chylomicron remnants. These particles are rapidly removed from the blood primarily by the liver. The liver has a complex chylomicron remnant removal system which is comprised of a combination of different mechanisms that include the low-density-lipoprotein receptor (LDLR) and the LDLR-related-protein (LRP). Furthermore, it has been suggested that there is a sequestration component whereby chylomicron remnants bind to heparan sulfate proteoglycans (HSPG) and/or hepatic lipase; this is then followed by transport to one or both of the above receptors for hepatic uptake. Over the years, a major concern has arisen about the association of chylomicron remnants and coronary heart disease (CHD) in man. Slow removal of chylomicron remnants, as reflected by a prolonged postprandial state, is now commonly observed in patients with CHD and those that have abnormal lipid disorders such as hypertriglyceridemia, familial hypercholesterolemia, familial combined hyperlipidemia and non-insulin-dependent-diabetes-mellitus. The present review will focus on (a) the details of the metabolic pathway (exogenous pathway) that describes the two-step processing of postprandial lipoproteins, (b) the role of the liver, the receptors, and the importance of efficient removal of chylomicron remnants from the blood circulation, and (c) the potential atherogenic effects of chylomicron remnants on the arterial wall.

2. INTRODUCTION

The postprandial state, in the context of the present review, refers to the period after food ingestion during which dietary lipid is transferred to the various parts of the body

via plasma lipoproteins. The two major plasma lipoproteins produced during the postprandial state are chylomicrons and chylomicron remnants. Many of the principle aspects of postprandial lipoprotein metabolism are now well understood and much attention has been directed towards unraveling the relationship of the process with the development of atherosclerosis (1,2,3,4,5). Two important sites of metabolism during the postprandial state are the liver (5) and the reticuloendothelial cells. There are a number of complex removal pathways that continue to be confusing and controversial (5,6,7). Abnormalities in any of these pathways in the liver may seriously impair efficient removal of these particles from the plasma leading to a longer residence in the blood which may cause damage to the arteries and may allow them alternatively to be deposited and accumulate in the arterial wall.

The present review will summarize the past and current state of knowledge on postprandial lipoprotein metabolism, the role of the liver, and the progress that has been made on the emerging concern about the relationship with atherosclerosis.

3. CHYLOMICRONS AND CHYLOMICRON REMNANTS

The metabolic route that describes the delivery of dietary triglyceride and cholesterol from the intestine to the peripheral tissues and the liver is referred to as the exogenous pathway of lipoprotein removal (8). Nascent chylomicrons synthesized by the intestinal mucosal cells (enterocytes) are triglyceride-rich lipoproteins by virtue of their higher triglyceride-to-cholesterol mass ratios, and consist primarily of the apolipoproteins (apo) B-48 and apoA-1 (9,10,11,12). The size of chylomicrons is dependent on the amount of dietary triglycerides and cholesterol and range from 80-1200 nm in diameter (9,13,14). They are secreted into the intestinal lymphatic capillaries; pass through the network of mesenteric lymphatic vessels into the thoracic lymph duct, and finally drain into the general blood circulation. It is in the thoracic lymph duct where chylomicrons acquire apoC-II and apoE (9,15,16).

In humans, primates and other higher mammalian species, the intestine is the exclusive site of synthesis of apoB-48; however, in the mouse and the rat, the liver is capable of synthesizing both apoB-48 and apoB-100 (17,18). ApoB-48 is, in fact, a truncated version of apoB-100 that is made by tissue-specific editing of the apoB-100 mRNA. (17). Interestingly, it has been demonstrated in the rat that chylomicrons are also produced during the fasting state (postabsorptive period), albeit with much lower triglyceride-to-cholesterol ratios; thus resulting in smaller very-low-density-lipoprotein (VLDL) sizes (19,20).

The function of apoB-48 has been the subject of much speculation and has yet to be clearly defined. Recently, in a mouse model where apoB-48 expression is lacking (chylomicron-deficient mouse), it was found that apoB-48 may be required as a critical surface component for the assembly of chylomicrons and thus be absolutely required for normal intestinal absorption of dietary lipid (21,22,23,24).

In tissue capillary beds, chylomicrons bind to the enzyme, lipoprotein lipase (LPL), fixed to the luminal surface of the capillary endothelial cells (25). LPL is activated by apoC-II and it lipolyzes the triglyceride in the chylomicrons (9,26,27,28,29). The lipolysis of the triglycerides yields free fatty acids which freely diffuse into cells where they are either oxidized for energy utilization or re-esterified for storage (15,30). ApoC-II and other redundant surface material (phospholipids and cholesterol) are lost in the process. The depletion of triglycerides results in the reduction of the size and the modified particles are then referred to as chylomicron remnants, or simply as 'remnants' (9). Chylomicron remnants are cholesteryl ester-enriched and they retain apoB-48 and apoE while the apoAs may be transferred to another plasma lipoprotein, high-density-lipoproteins (HDL) (31,32,33). The liver is the major organ that removes remnants from the blood circulation (5,34).

Figure 1 illustrates the exogenous pathway and its relationship with the endogenous pathway; hepatic production of VLDL and conversion to low-density-lipoproteins (LDL) (15,35,36), and the reverse cholesterol pathway, the removal of cholesterol from peripheral tissues by HDL (37,38). The endogenous pathway and the reverse cholesterol pathway are not the subjects of the present review but related specific aspects are worth mentioning. Elevated plasma LDL concentration is a risk factor for coronary heart disease (CHD) (39,40) while elevated plasma HDL is associated with protection (41). All lipoproteins are in dynamic interactions with each other in the plasma and this promotes the exchange of lipids among different lipoproteins mediated by the enzyme, cholesteryl ester-transfer-protein (CETP) (42,43,44).

Animals fed high cholesterol-enriched diets produce remnant-like lipoproteins called beta-very-low-density-lipoproteins (beta-VLDL) (45). These are small cholesterol-rich, apoE-containing particles of intestinal and hepatic origin (45). The precise steps involved in the formation of beta-VLDL during the postprandial state are still undefined. Beta-LDL is also found in the plasma of patients with type III hyperlipoproteinemia, a condition where there is hypercholesterolemia and hypertriglyceridemia (46,47). Previous studies suggest that beta-VLDL is highly atherogenic based on evidence that the uptake of these lipoproteins cause massive cholesterol accumulation and foam cell formation in macrophages (48,49,50,51,52).

4. METHODS TO DETERMINE THE REMOVAL OF CHYLOMICRONS AND REMNANTS FROM THE PLASMA

The rate of plasma removal of postprandial lipoproteins is usually assessed to determine how efficient an individual's metabolism is during the postprandial state (3,4,53,54,55) and this has become the basis for

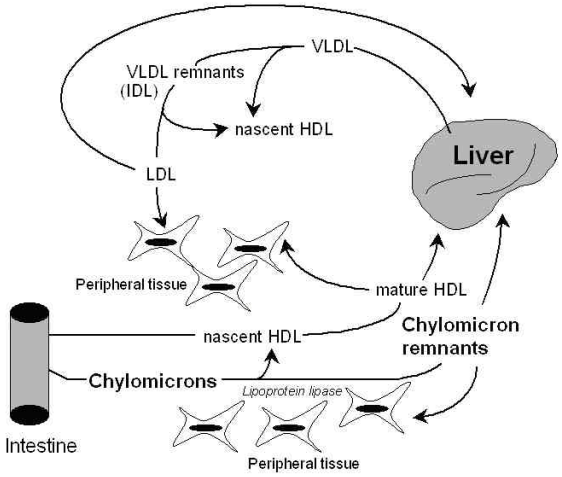

Figure 1. The exogenous and endogenous pathways of plasma lipid transport. The exogenous pathway comprises the synthesis of chylomicrons from dietary lipids absorbed by the intestine. The chylomicrons are secreted into the blood circulation and are converted to chylomicron remnants by lipolysis through interaction with tissue capillary endothelium-bound LPL. The chylomicron remnants are primarily removed from the circulation by the liver while a small percentage will be taken up by peripheral tissues (bone marrow, adrenals, arteries, etc.). The endogenous pathway begins with the synthesis of VLDL which is converted to VLDL remnants (IDL) and LDL by LPL. The VLDL remnants are removed by the liver while LDL are removed by steroidogenic tissues, other tissues with a requirement for cholesterol, and the remainder by the liver. HDL are formed both by the intestine and from the liver, or from excess surface material from chylomicrons and VLDL. The HDL pathway is not discussed further for the sake of simplicity. Abbreviations: HDL, high-density-lipoproteins; IDL, intermediate-density-lipoproteins; LDL, low-density-lipoproteins; VLDL, very-low-density-lipoproteins.

experimental testing of postprandial lipoprotein metabolism. In normolipidemic individuals, the postprandial period is about 4-6 hours, but in individuals with certain dyslipidemic conditions, the postprandial period may be increased beyond 6 hours (3,56). The two common tests make use of an intravenous bolus injection of lipid and an oral fatty test meal.

4.1. Intravenous bolus injection

In animals, studies done as early as 1967 (57,58), labeled chylomicrons were injected intravenously. The disappearance of the label in the plasma over time is measured (59,60,61,62,63). Redgrave and his colleagues have devised an artificial chylomicron-like emulsion, prepared from mixtures of lipid in which double radiolabels can be inserted into the cholesteryl ester and cholesterol separately (64,65,66). The removal of chylomicrons-chylomicron remnants is extremely rapid. The half-life has been reported to be 10-20 min in mice (67,68), 20-25 min in rabbits (63) and 12-18 min in rats (69,70). Recently, Redgrave's laboratory has devised a non-invasive method in which radiolabeled emulsions are introduced intravenously but instead of obtaining blood plasma samples, breath samples (expired CO_2) are collected and analyzed for appearance of the radiolabel (66,71). This test makes use of the fact that the expired air would contain radiolabeled CO_2 derived from oxidative metabolism of the fatty acid produced from hydrolysis of the radiolabeled (^{14}C-)cholesteryl ester. A stable isotope, i.e., ^{13}carbon, can be used instead of ^{14}carbon, to reduce radioactivity exposure and may prove useful for studies in humans (72).

4.2. Oral fat test meal (fat tolerance test)

In humans, a standardized oral fat test meal (fat tolerance test) is given to evaluate the removal of postprandial plasma lipid/lipoproteins (73,74,75,76). Although triglyceride and cholesterol concentrations can be measured in the density<1.006 g/ml fraction of postprandial plasma, the inherent problem is the contamination from the endogenous hepatic-derived lipoproteins, VLDL and their remnants. To circumvent this, chylomicrons and chylomicron remnant concentrations can be measured by analytical SDS-PAGE of proteins from delipidated postprandial plasma (77,78) or determined by Western blotting using anti-apoB antibodies (79). The problem with Western blotting is that the anti-apoB antibodies do not distinguish apoB-48 from apoB-100 (80). More recently, several Japanese groups (81) have reported raising a monoclonal antibody specifically recognizing apoB-48 only and not apoB-100. The two step procedure of Uchida *et al* (81) of removing apoA-1-containing lipoproteins followed by use of the monoclonal antibody has been put forward as a measure of both endogenous and exogenous remnants since it still retains some apoB-100-containing lipoproteins.

The fat-soluble vitamin A, retinyl palmitate, can be added as a supplement to the oral fat test meal (82), allowing the retinyl palmitate in postprandial plasma to serve as a marker for chylomicrons and chylomicron remnants. Further refinement of this method by Berr and Kern (83) demonstrated there was none or very little exchange of retinyl palmitate among different lipoprotein fractions early in the test. Studies by Cohn *et al* (74,84,85), however, highlighted the problem of retinyl palmitate exchange from the intestinally-derived to the hepatic-derived lipoproteins at late time points after 6 hours. At least within a limited time period after ingestion of the fatty test meal, the retinyl palmitate works well but still does not discriminate chylomicrons from remnants.

The recent interest of using stable non-radioactive tracer isotopes may improve evaluation of lipoprotein metabolism in man. Details of the techniques, their applications, their advantages and drawbacks in lipoprotein kinetic studies have been reviewed by Schaefer *et al* (86) and Watson (87). Briefly, the most common isotopes used is D_3-leucine for the endogenous labeling of apolipoproteins while ^{13}carbon-labeled lipids are used in oral or intravenous administration. These isotopes are measured by gas chromatography/mass spectrometry. It has been argued that stable isotope experiments give different result to traditional radioisotope experiments,

however, Ikewaki *et al* (88) found a good correlation for apoA-1 kinetics between the two methods for results from eight individuals. Dermant *et al* (89) have shown the feasibility of using the stable isotopes for the measurement of human apoB *in vivo*. The estimation of the fractional synthetic rate of apolipoproteins and lipids has been studied using this technique (90,91). The potential of using stable isotopes in studies of postprandial lipoproteins, although not yet explored, should not be underestimated. More recently, Welty *et al* (92) infused D_3-leucine to normal subjects during the fed state after consumption of a western diet and they found that VLDL apoB-100 was removed from the plasma at a faster rate than triglyceride-rich lipoprotein B-48.

In summary, there is no single, precise test that easily allows the study of chylomicron remnant metabolism in humans, and this has impeded progress in this field.

5. THE ROLE OF THE LIVER

The duration of the postprandial period depends on the balance between gastric emptying and the rate of chylomicron remnant uptake by the liver. Impairment in the removal pathways in the liver may potentially lead to decreased rate of uptake of chylomicron remnants, thus leading to increased residence time of these particles in the blood circulation. This prolonged postprandial period has been the subject of a number of investigations and some now argue that abnormal metabolism of postprandial triglyceride-rich lipoproteins has an atherogenic (3,93).

Consideration must be given to the morphology of the liver as it is paramount to the metabolism of chylomicron remnants. The liver has a complex architecture made of different cell types. Macromolecules (lipoproteins, albumin, fibrinogen, etc.) entering the liver via the hepatic portal vein flow into the liver sinusoids (irregularly dilated vessels lined by endothelial cells) (94). The endothelial cell layer is separated from the underlying hepatocytes by the subendothelial space called the space of Disse. The openings within the endothelial cell, fenestrae, are grouped in clusters forming 'sieve plates'. These 'sieve plates', as suggested by Wisse *et al* (95), act as a filter for the selective entry or secretion of macromolecules.

The sieving phenomenon probably restricts the size of chylomicron remnants and other lipoproteins moving from the sinusoids into the space of Disse (96). In fact, small chylomicrons (<100 nm) may pass through the sieve while larger particles may not. Previously, Windler *et al* (97) found that large chylomicron remnants were removed from plasma by the liver at a greater rate than small chylomicron remnants; however, large chylomicron remnants were internalized more slowly than small chylomicron remnants. They proposed that the large particles were modified before receptor-mediated endocytosis while the small particles bound directly to the receptors for endocytosis.

The hepatocytes are the major cells in the liver that take up chylomicron remnants; however, there are other cell types of which their role in the catabolism of chylomicron remnants remains unclear. The Kupffer cells (localized phagocytic cells) are found irregularly in the liver sinusoids and the space of Disse; these cells are specialized scavengers (98). The fat-storing hepatic stellate cells (Ito cells) are found in the space of Disse scattered among the hepatocytes and an essential function is the accumulation of exogenous vitamin A as retinyl esters (94,99). The quantity of lipoproteins removed by these cells is small compared to hepatocytes (100).

Receptor-mediated mechanisms are the predominant pathways chylomicron remnants are taken up by the hepatocytes (5,101,102). It is now established that apoE is important for the interaction of chylomicron remnants and the receptors. The receptors for chylomicron remnants and how chylomicron remnants are internalized in the liver, however, is still a subject of controversy, despite over two decades of accumulating knowledge. Different molecules have been described as receptors for chylomicron remnants and their potential roles are summarized below.

5.1. Receptors for chylomicron remnants

5.1.1. LDL receptor

Although apoB-100 (in LDL) was initially ascribed as the ligand (15), apoE (in remnant lipoproteins) was later identified as a ligand for the LDL receptor (61,62,103). There are three major isoforms of apoE referred to as apoE2, apoE3 and apoE4. ApoE3 and apoE4 bind normally to the LDL receptor but not apoE2 (104,105). ApoE-containing lipoproteins have a higher affinity for the LDL receptor than LDL and the affinity increases with the number of molecules of apoE per particle (103,106).

The issue of the LDL receptor pathway being the major mechanism for chylomicron remnant uptake was debated for many years because of conflicting observations in different laboratories. Initially, Kita *et al* (63) found that the plasma removal of radiolabeled chylomicrons was normal in the WHHL rabbits, an animal model of genetically inherited LDL receptor deficiency. A similar observation was reported in humans (107). On the other hand, Nagata *et al* (108) and Choi *et al* (109) found that the LDL receptor was the major pathway for chylomicron remnant uptake in the mouse and the rat *in vitro* and *in vivo*. Other laboratories (110,111,112) subsequently reported that there is impaired plasma chylomicron remnant removal in the absence of LDL receptors *in vivo*.

More recently, our laboratory confirmed the importance of the LDL receptor in the mouse (113,114). The concentration of chylomicron remnants in the plasma may be a critical factor affecting the hepatic removal of chylomicron remnants (114). In the absence of LDL receptors (LDLR-knockout mouse), small concentrations of radiolabeled chylomicron remnants are removed efficiently by the liver while relatively larger concentrations were not. The discrepancies in remnant removal observed previously in different laboratories (63,67,79,107,111,115) may be explained by the variability of different remnant

concentrations used, a factor that was difficult to assess in those studies.

5.1.2. LDL receptor-related-protein/alpha$_2$-macroglobulin receptor

The LRP, or alpha$_2$-macroglobulin receptor is a multi-ligand receptor that binds apoE-containing remnant lipoproteins and other proteins, i.e., alpha$_2$-macroglobulin, lactoferrin, lipoprotein lipase and tissue-type plasminogen activator (t-PA) (116,117,118,119,120). The LRP is a larger protein (~660 kDa) than the LDL receptor (~120 kDa) and has a number of similar structural motifs to the LDL receptor. In fact, the LRP was the progenitor receptor from which the LDL receptor, the VLDL receptor, gp330 and a number of other molecules evolved from (120). They are often referred to in the literature as belonging to the LDL receptor gene family (120). Unlike the LDL receptor, the regulation of LRP numbers is not affected by the change in intracellular lipid content (120). Enrichment of beta-VLDL with exogenous apoE enhances LRP-mediated uptake (121,122). The production of the LRP is dependent on the 39-kDa receptor-associated-protein (RAP) (123,124,125). The RAP is a molecular chaperone that assists in the proper assembly of the LRP within the endoplasmic recticulum and it transports the LRP to the plasma membrane (126,127). The RAP remains associated with the LRP on the cell surface (127). Interestingly, exogenously added RAP has been shown to be an effective antagonist for ligand interaction with the LRP (128,129) but the mechanism is not clear.

In cell culture experiments, LRP mediated uptake of lipoproteins requires addition of exogenous apoE. Eventually, it has been demonstrated that the LRP is a physiological pathway for the plasma removal of chylomicron remnants *in vivo* (68,115,119,130). In the LRP knockout mice, LDL receptor levels increase enough to maintain normal lipid levels; and only when both pathways, the LRP and the LDL receptor, are absent is there accumulation of remnant-like lipoproteins in these mice given a normal diet (131). The LRP appears to be a less efficient pathway for removal of lipoproteins than the LDL receptor. In the LDLR-knockout mouse, while plasma removal of intravenous injected chylomicrons was normal, chylomicron remnant uptake by the hepatocytes was slow (67,115). Interestingly, our studies in isolated perfused livers from LDLR-knockout mice showed decreased removal of remnants as concentration increased (114) and this is associated with a very different pathway for processing apoE-containing remnants. As pointed out earlier, the concentration of remnants used by Herz *et al* (115) and Mortimer *et al* (67) was sufficiently small for effective liver uptake in the absence of LDL receptors.

5.1.3. Heparan sulfate proteoglycans and hepatic-localized apoE

Heparan sulfate proteoglycans (HSPG), an extracellular matrix protein, belong to the family of glucosaminoglycans and are abundant in the space of Disse on the endothelial and hepatocyte surface (132). Attached to the HSPG is apoE that is synthesized by the hepatocytes (133). Mahley and his colleagues (101,134,135,136) proposed that HSPG and hepatic-localized apoE are components of a pathway of remnant uptake referred to as 'secretion-capture'. This pathway takes place in the space of Disse where remnants initially bind to HSPG. Then, the remnants are modified by the attachment of additional apoE from HSPG. The remnants attached with additional apoE, referred to as 'apoE-enriched remnants' are transported by the HSPG to the LRP for uptake by the hepatocytes. Alternatively, it has been suggested that HSPG themselves are capable of facilitating remnant uptake (137,138).

Though apoE is important, remnants may under normal circumstances contain enough apoE (obtained from the plasma) for uptake in the liver by the LDL receptor although it is still inconclusive whether this is enough for uptake by the LRP. Studies by Linton and Fazio (139,140,141,142,143) have used bone marrow transplantation (BMT) of apoE-secreting macrophages to investigate the threshold of apoE concentration required for normal removal of remnants. They demonstrated that BMT is an effective tool to restore normal lipid levels in apoE-knockout mice but not in apoE/LDLR-double-knockout mice (139). They argue that hepatocyte-secreted apoE is needed for the 'secretion-capture' pathway where uptake is by the LRP whereas macrophage-secreted apoE (derived from BMT) is ineffective despite the presence of abundant apoE in the space of Disse. In other words, macrophage-secreted apoE is different from hepatocyte-secreted apoE in its interaction with remnants and the LRP. Alternatively, it may be that excessive concentrations of apoE in the space of Disse may inhibit access of remnants to bind to the LRP. Later, van Dijk *et al* (144), using adenovirus delivery of apoE gene, found that higher than normal levels of serum apoE was required to restore normal lipid levels in apoE/LDLR-double-knockout mice which suggest that the LRP requires a very high serum level of apoE. However, in our studies, remnants prepared in eviscerated animals contain sufficient apoE for rapid uptake by either pathway (145). The particles may not be representative of what is found *in vivo*. Our recent studies demonstrated that the removal of remnants by isolated perfused livers from apoE-knockout mice was normal compared to wild-type mouse livers (Yu *et al*, unpublished observations). Our results thus suggest that hepatic secretion and localization of apoE is not critical for remnant uptake by the liver and thus, may not be required for LRP-mediated uptake, at least at moderate levels. Even particles containing no apoE can be rapidly removed by the livers of normal mice. Thus, apoE may be acquired either in the circulation or the liver and the secretion capture model may not be completely accurate.

Increased production of apoE in the serum has been successfully achieved either by transgenes (146) or adenovirus (144,147,148) and this results in accelerated removal of remnants from the plasma. This strongly suggests that the rate at which remnants are removed by the liver is dependent on the rate at which the remnants acquire apoE in the periphery. It does not, however, necessarily imply that the rate of remnant removal is a function of apoE concentration because very high levels of apoE have been demonstrated to inhibit LPL activity (149). Resolution of this controversy may relate to the observation

that very high levels of apoE inhibit LPL thus slowing remnant formation in the *in vivo* studies.

5.1.4. Hepatic Lipase

The major function of hepatic lipase appears to be the lipolysis of VLDL remnants (IDL) to form LDL and perhaps to convert HDL_2 to HDL_3 (28) but over the last decade, interest has arisen about the possible role of hepatic lipase in the uptake of lipoproteins containing apoB (VLDL remnants, LDL, chylomicron remnants). Several studies demonstrated that inhibition of hepatic lipase activity led to an impairment of chylomicron remnant uptake by the liver (150,151,152,153,154). Amar *et al* (155) found that inactive hepatic lipase (elimination of lipolytic activity) may be a co-ligand for the binding of remnants and cell surface receptors and/or HSPG. In contrast, Choi *et al* (156) demonstrated in CHO cells transfected with hepatic lipase that chylomicron remnant uptake remained at normal levels but interestingly, LDL uptake was increased several-fold. The excess hepatic lipase did not stimulate enhanced uptake of chylomicron remnants which suggest that hepatic lipase alone was insufficient but may act in tandem with other molecules, such as HSPG and apoE. In the isolated perfused rat liver, Shafi *et al* (154) reported that antibodies against hepatic lipase inhibited remnant uptake and furthermore, there was decreased binding to the cell surface but increased endocytosis.

In rats and rabbits, hepatic lipase remains localized in the liver due to strong binding to the extracellular matrix of the endothelial cells but in the mouse there is poor anchoring of hepatic lipase to the extracellular matrix (157,158,159). Hence, the mouse has less hepatic lipase in its liver but instead has hepatic lipase in the plasma, whereas rat and rabbit hepatic lipase remains localized in the liver and very little in plasma. The plasma removal of chylomicron remnants in the normal mouse is still very efficient and does not differ significantly from that in mice lacking hepatic lipase (160) and this suggests that hepatic lipase is not a critical factor although its inhibition by anti-LPL antibodies did slow remnant removal in both mice and rats.

5.1.5. Phospholipolysis-dependent pathway

Borensztajn and co-workers (161,162,163,164) proposed that phospholipolysis of chylomicron remnants is a prerequisite for uptake in the liver and is independent of apolipoproteins. Phospholipase-treated chylomicrons or hepatic lipase-treated chylomicrons were removed from plasma by the liver efficiently. More recently, they have shown that chylomicron remnants devoid of apolipoproteins made by treatment with trypsin and/or hepatic lipase were removed by the liver efficiently in wild-type and apoE-knockout mice (164). They proposed that the remnants initially bind to hepatic lipase and/or phospholipase whereby hydrolysis of phospholipids and triglycerides occur and modify the particles for subsequent endocytosis. It is still unclear whether or what other molecules are involved in the hepatic uptake of these 'phospholipolyzed remnants'. In contrast, recent studies in our laboratory have demonstrated impaired removal of trypsin-treated chylomicron remnants by the isolated perfused liver that lacks apoE (Yu *et al*, unpublished observations).

5.1.6. Other molecules

Plasma LPL, as initially suggested by Felts and his colleagues (165), might be acquired by remnants during their formation and thus serve as the moiety that mediates hepatic uptake. Studies by a number of laboratories have demonstrated that lipoprotein lipase enhanced chylomicron remnant uptake by the LRP *in vitro* and by the liver *in vivo* (102,166,167,168,169) which strongly support the role of lipoprotein lipase in the hepatic uptake of remnants. Furthermore, dimers of lipoprotein lipase have been found in triglyceride-rich lipoproteins isolated from post-heparin plasma (170). It seems possible that LPL dimers may associate with remnants post-lipolysis in the plasma before entering the liver but it is inconclusive whether these remnants attached with LPL dimers would be able to enter the space of Disse through the endothelial fenestra or that further modifications of these remnants are required in the liver. However, it should be noted that there is little or no LPL molecules present in the liver and that in studies *in vivo* only there appears to be less than one molecule per particle associates with remnants (171,172).

Van Berkel and his colleagues (173,174,175) have hypothesized that there is a non-LDL receptor, non-LRP pathway for remnants that they call the "lipoprotein-remnant receptor" which is insensitive to competition with the receptor-associated-protein (RAP), $alpha_2$-macroglobulin and beta-VLDL and apparently operates when the concentration of LDL receptors is very low. This receptor, however, is inhibited by lactoferrin, a milk protein, that is also a ligand of the LRP.

5.2. Processing and uptake of chylomicron remnants in the liver

Unlike the simple model of LDL uptake by the LDL receptor pathway, chylomicron remnant uptake by the liver is a more complicated process, a system that possibly involves complex regulation of different pathways that are influenced by varying metabolic states.

It would appear that even with rapid removal, there are rapid and slower internalization pathways. The slower uptake pathways represent 'sequestration' components which bind the remnants to the cell surface (hepatocytes and endothelial cells) in the space of Disse. In other words, sequestration enables the rapid removal of remnants and their retention on the cell surface before internalization by the slower pathways. The model for the hepatic uptake of chylomicron remnants we propose based on our and other observations elsewhere is illustrated in figure 2. In this model, chylomicron remnants enter the space of Disse where they may bind to different proteins simultaneously. The receptors or proteins that the remnants bind to are dependent on ligand affinity. Our studies suggest that the LDL receptor is a rapid high-affinity high-capacity pathway and it is the most important pathway for hepatic removal of chylomicron remnants (109,113,114) when it is present. The LRP is very likely the second most important but it appears to be a slower low-affinity

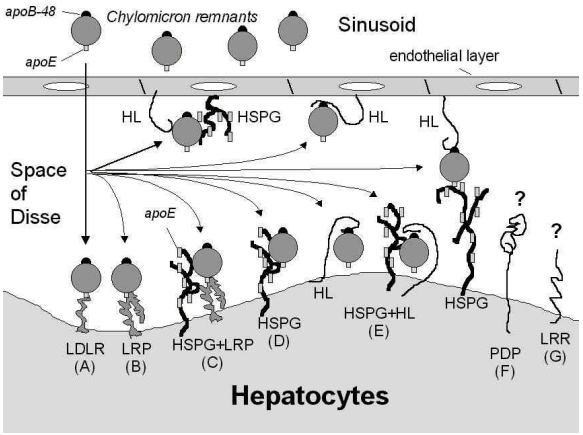

Figure 2. Pathways of chylomicron remnant uptake by the liver. Chylomicron remnants entering the liver pass from the sinusoids, then through fenestrae in the endothelial layer and into the space of Disse. A large number of remnants will be taken up directly by the LDL receptor (A) because it is the rapid high-affinity high-capacity pathway. Simultaneously, a smaller number of remnants may (i) be taken up directly by the LRP (B) which is a slow low-affinity low-capacity pathway, (ii) be sequestered by heparan sulfate proteoglycans and acquire additional apoE and then be transferred to the LRP (C) for uptake, (iii) be taken up directly by heparan sulfate proteoglycans (D), (iv) be sequestered by heparan sulfate proteoglycans and/or hepatic lipase (E) and then be transferred to the LDL receptor or LRP for uptake, (v) be taken up by the phospholipolysis-dependent pathway (F) and/or lipoprotein-remnant receptor (G). Abbreviations: HL, hepatic lipase; HSPG, heparan sulfate proteoglycans; LDLR, low-density-lipoprotein receptor; LRP, LDL receptor-related-protein; LRR, lipoprotein-remnant receptor; PDP, phospholipolysis-dependent pathway.

pathway and, at least based on our preliminary data, leads to slow internalization. It is, however, inconclusive as to whether HSPG and hepatic-localized apoE are necessary for the LRP pathway (7). Furthermore, studies in mice and men suggest that the LDL receptor may be up-regulated if LRP is down-regulated (130,131,176,177). HSPG alone may act as a receptor capable of internalizing remnants (137,138) or may act in tandem with hepatic lipase to bind remnants before transporting the remnants to the LDL receptor, the LRP or other receptors. The combination of the LRP, HSPG and/or hepatic lipase may be the sequestration component. The other pathways (lipoprotein-remnant receptor and phospholipolysis-dependent pathway), as mentioned earlier, are still not clearly established but possibly play less important roles unless the other two systems are absent and/or saturated, as occurs in the apoE/LDLR-double knockout mice where some form of slow remnant removal must be occurring.

Why are there separate pathways that operate differently from each other? The common feature of all these receptors seems to be the rapid removal of remnants from the blood circulation. Hence, it is plausible that the system has evolved to keep remnants at a minimal concentration in the plasma so as to avoid damaging effects on the arterial wall.

6. THE METABOLISM OF CHYLOMICRON REMNANTS IN THE ARTERIAL WALL AND ATHEROSCLEROSIS

Donald Zilversmit, in 1979, proposed that chylomicrons or chylomicron remnants *per se* may cause atherosclerosis (1). The influx of lipoproteins from the plasma into the arterial wall is likely a prerequisite for any atherosclerotic development to occur (178,179). The predominant cells in the normal arterial wall without atherosclerotic lesions are endothelial cells and smooth muscle cells (179,180) while in lesioned arterial wall, the additional cells are the macrophages (179,181,182). It is generally accepted that gross changes to the arterial tissue take place when lipoproteins accumulate and this may promote atherogenesis (178,183,184,185,186).

Currently, the most widely accepted hypothesis of atherogenesis currently is the 'oxidized LDL' proposal (187). In the arterial wall, it is proposed that LDL undergoes oxidative modification and there is production of toxic oxysterols and other oxidized lipid and lipoprotein products. The macrophage secretes oxidizing agents such as superoxide, hydrogen peroxide and hypochlorous acid (188) that oxidize LDL (189,190). There is now a vast body of knowledge regarding the atherogenic effects of oxidized LDL on the arterial tissue *in vivo* and *in vitro;* however, there is still a paucity of information about the potential atherogenic effects of chylomicron remnants and whether they are atherogenic at all. It is thus appropriate to discuss the metabolism of chylomicron remnants in the cells in the arterial wall and lesions.

6.1. Endothelial cells

The endothelial layer of the arterial wall is a barrier to the plasma compartment and only permits certain macromolecules to pass into the subendothelial layer and the medial layer (185,191). It probably differs from the liver sinusoidal endothelial layer by the absence of fenestra. It was initially thought that injury to the endothelial layer that resulted in the opening of intercellular pores between tight junctions was what permitted lipoproteins to move from the plasma compartment to the arterial wall (179). Palade *et al* (192), however, found that molecules as large as peroxidase were unable to move through pores between tight junctions, but instead were internalized by the endothelial cells and crossed the cell cytoplasm by transcytosis. Transcytosis is a mechanism whereby macromolecules are transported from one end of the cell to the other; bypasses the lysosomal vesicles (thus avoiding degradation) and are then exocytosed into the subendothelial layer (193,194). This mechanism appears to be non-specific and only size seems to be the restricting factor for what can be transcytosed (192). The concentrations in the plasma compartment and the arterial wall may affect the transcytosis of particles. In such a way, the endothelial layer may, in fact, act like a semi-permeable osmotic barrier. Simionescu *et al* (195,196) showed that LDL was trancytosed across the arterial endothelial layer in

rats and rabbits. They showed vesicles existed in endothelial cells that faced the plasma compartment, and the sizes of these vesicles were up to 70 nm. Almost 90% of LDL transported across the endothelial layer were observed in these vesicles while 10% were in lysosomes.

In early studies, Newman and Zilversmit (197,198) demonstrated in animals that radiolabeled cholesterol in the diet was taken up by atherosclerotic lesions. Previously, it was thought that chylomicron remnants were unable to cross the endothelium and penetrate the arterial wall because chylomicron remnants are larger particles (~40 nm) than LDL (~10 nm) (191). Later, Mamo *et al* (199,200) obtained chylomicrons from rat lymph and these were injected intravenously into the rabbit where they were taken up by the normal and lesioned arterial wall. These observations proved that remnants can penetrate the arterial wall and further supported Zilversmit's hypothesis that remnants are potentially involved in the pathogenesis of atherosclerosis (1). Rapp *et al* (201) found remnant- and VLDL-sized particles in atherosclerotic lesions but found only apoB-100 in the particles.

During high fat-diet-induced development of atherosclerotic lesions, the expression of cell adhesion molecules (adhesive glycoproteins) are induced on the endothelial cell surface *in vivo*. In rabbits, vascular cell adhesion molecule-1 (VCAM-1) is produced after 4 days of hypercholesterolemia (202,203). The presence of this and other adhesion molecules such as intracellular adhesion molecule (ICAM), platelet-endothelial cell adhesion molecule (PECAM, E-selectin and P-selectin (204,205,206) that gives rise to the phenomenon called 'monocyte rolling' whereby blood monocytes roll, tether and then bind to the adhesion molecules (207,208,209,210) on the endothelial surface (179). The monocytes eventually migrate into the subendothelial space by 'squeezing' through the tight junctions (211,212,213). There is some evidence that suggest endothelial expression of adhesion molecules is affected by lipoproteins (214,215).

Previously, Hennig *et al* (216) found that remnants of triglyceride-rich lipoproteins from the serum of hypertriglyceridemic subjects were toxic to endothelial cells and seriously damage the endothelial barrier function. Along similar lines, chylomicron remnants in prolonged contact with the arterial endothelium may also be capable of damaging the endothelial barrier function.

6.2. Smooth Muscle Cells

Smooth muscle cells are located in the medial layer and they form the bulk of the arterial wall (180). The role of smooth muscle cells is seen as less important than macrophages; however, certain relevant aspects must be noted. Smooth muscle cells migrate to the intimal layer during atherosclerosis development where they then multiply (179). The increased numbers of smooth muscle cells causes growth of the lesion and this may reduce the size of the blood vessel lumen, thus causing blood flow obstruction. A small percentage of smooth muscle cells in the intimal layer are converted to foam cells (179). It is likely that retention and overaccumulation of lipoproteins in the intimal layer somehow causes the smooth muscle cells to migrate from the medial to the intimal layer and in addition, change their phenotypic state from that of a 'quisecent state' to a 'synthetic state' (180). The uptake of lipoproteins is the probable means by which smooth muscle cells are converted to foam cells by pathways similar to that in macrophages.

Cultured arterial smooth muscle cells are capable of efficient uptake of lipoproteins. In swine arterial smooth muscle cells, LDL uptake is mediated via the LDL receptor (217). More than a decade ago, Floren *et al* (218) showed that chylomicron remnants were endocytosed by human arterial smooth muscles and increased cellular cholesterol content 3-fold more than LDL. The LDL receptor was the major pathway for chylomicron remnant uptake and this is consistent to that found in cultured skin fibroblasts and hepatocytes (218,219,220).

If the LDL receptor is important, then down-regulation of the expression and activity of this receptor may reduce remnant uptake. Limited numbers of LDL receptors may still be able to efficiently endocytose LDL but not remnants. In other words, decreased expression and activity of the LDL receptor may have a greater impact on remnant catabolism than on LDL catabolism. This may lead to increased retention of remnants in the extracellular space.

6.3. Macrophages

The macrophage's primary role in atherosclerosis is believed to be the removal of lipid containing particles, lipoproteins and lipoprotein aggregates from the arterial wall (121,179,182). As mentioned earlier, monocytes migrate from the plasma compartment into the subendothelial space by 'rolling', adhesion and 'squeezing through tight junctions'. In the subendothelial space, the monocytes are activated and differentiate into macrophages. Macrophages are phagocytes but they are also capable of internalizing particles by classical receptor-mediated endocytosis. The LDL receptor, scavenger receptors, LRP and other recently discovered receptors including CD36 and macrosialin are expressed by macrophages (120,221,222,223). The uptake of lipid converts macrophages into foam cells, the hallmark feature of atherosclerotic lesions. The mechanism of foam cell formation requires subversion of normal regulation of intracellular cholesterol homeostasis. The regulation of cholesterol, as delineated by Brown and Goldstein (121), involves two enzymes, HMG CoA reductase and ACAT, and the LDL receptor gene. HMG CoA reductase is the rate-limiting enzyme in the synthesis of cholesterol and their activity is regulated by the free cholesterol pool. When the free cholesterol pool expands, as a result of increased LDL uptake, HMG CoA reductase is inhibited. The LDL receptor gene is down-regulated and this decreases the number produced thus decreasing the amount of LDL-cholesterol taken up. ACAT esterifies free cholesterol to cholesteryl ester and these appear as lipid droplets in the cytoplasm. An abnormally high number of cholesteryl ester droplets in the macrophage give a distinct

'foamy' appearance; hence, lipid-laden macrophages are called foam cells. In foam cell formation, there is continual uptake of lipoprotein despite reduced LDL receptor levels and ACAT activity is up-regulated, thus causing increased production of cholesteryl ester (121). One of the requirements for lipoproteins to be atherogenic is whether they can cause foam cell formation. The uptake of LDL in its native form does not cause foam cell formation, instead, oxidation of LDL gives rise to atherogenic particles (187). In addition, the uptake of beta-VLDL also causes foam cell formation. Oxidized LDL and beta-VLDL uptake have been shown to have minimal effect on HMG CoA reductase activity but enhanced ACAT activity (esterification of cholesterol) compared to native LDL. A study by Yu and Mamo (224) in rabbit lung macrophages showed that regulation of HMG CoA reductase and ACAT activity after chylomicron remnant uptake was similar to that induced by LDL. This is in contrast to the findings of Ellsworth *et al* (225) who found that cholesterol synthesis was increased and cholesterol esterification was decreased in murine J774 macrophages. Previously, Georgopoulos *et al* (226) found that postprandial triglyceride-rich lipoproteins from diabetic subjects stimulate esterified cholesterol accumulation in the THP-1 macrophage cell line and more recently, it was demonstrated that chylomicron remnant uptake caused lipid accumulation in mouse peritoneal macrophages and human monocyte-macrophages *in vitro* by both LDL receptor-dependent and -independent mechanisms (145,227).

Though LDL metabolism in macrophages is well established (8), the same may not be said for chylomicron remnants. There are still conflicting observations about the chylomicron remnant uptake mechanisms. It has been previously demonstrated in several laboratories that the LDL receptor is the major mechanism for chylomicron remnant uptake in macrophages from different species (225,228,229,230). The same receptor also transports beta-VLDL (225,228,229,230). Although the LDL receptor transports LDL and beta-VLDL, however, there are differences in their endocytic patterns. In mouse peritoneal macrophages, beta-VLDL were transported to widely distributed and perinuclear vesicles whereas LDL were transported to perinuclear lysosomes (231,232). This difference may explain why beta-VLDL is degraded by macrophages at a slower rate than LDL; furthermore, it may explain why beta-VLDL is a more potent stimulator of ACAT activity than LDL is (48,233). It was also noted that intestinally-derived beta-VLDL were found in the widely distributed vesicles but hepatic-derived beta-VLDL was found in the perinuclear lysosomes like those LDL were found in (232). These observations suggest that even though the LDL receptor is the major mechanism for chylomicron remnant and LDL uptake in normal macrophages, the processing differs depending on the specific lipoprotein. This may help explain the mechanism of chylomicron remnant-induced foam cell formation (145,227).

There are also reports of other possible receptors. Goldstein *et al* (48) proposed the 'beta-VLDL receptor' based on observations that beta-VLDL uptake persisted despite LDL receptor down-regulation. In contrast, van Lenten *et al* (49) found that there was no change in beta-VLDL uptake in monocyte-macrophages from LDL receptor-deficient individuals compared to normal individuals. Mamo *et al* (234) found a 'phagocytic-like' pathway in rabbit lung macrophages, possibly initiated by binding to a 43 kDa protein (Mamo, personal communication). Macrophages also have LRP that can take up remnants (145,235,236). More recently, Gianturco and her colleagues (237,238,239) reported the cloning of a 'apoB-48 receptor' that binds triglyceride-rich lipoprotein remnants.

7. POSTPRANDIAL LIPOPROTEINS AND ATHEROSCLEROSIS IN MAN

In the 1950s, it was reported that patients with coronary heart disease (CHD) have increased postprandial triglycerides in their plasma due to a prolonged postprandial period (240). Josef Patsch and his co-workers suggested that a poor ability to remove postprandial lipid is likely to increase the risk of atherosclerosis because it promotes the transfer of cholesteryl ester from LDL and HDL to triglyceride-rich lipoproteins and remnants converting them to potentially atherogenic particles (4,75,93,241). There are now an increasing number of studies on the role of postprandial lipoproteins in atherosclerotic disease.

An early study in man was done by Simons *et al* (242) who examined fasting and postprandial plasma using a semi-quantitative assay for apoB-48 and apoB-100. A higher apoB-48:apoB-100 ratio was associated with CHD. In a similar study, Simpson *et al* (243) used the retinyl palmitate test to investigate patients with coronary atherosclerosis and they found the disease positively correlated with prolonged postprandial lipemia and low HDL levels irrespective of LDL concentrations. More surprising was that the retinyl palmitate continued to persist in the plasma for 24 hours after the oral fatty meal but this may have been caused by exchange to endogenous lipoproteins at late time points (74,84,85). Coronary bypass patients also have impaired plasma removal of chylomicron remnants despite normal lipoprotein lipase activity (244).

Individuals with CHD who are hypertriglyceridemic have slower plasma removal of chylomicron (245) and the progress of the disease over a 5-year period positively correlated with lipoproteins containing apoB-48 (246). Patsch *et al* (75) found that age, postprandial plasma triglyceride and fasting plasma apoB provided the greatest sensitivity and specificity for predicting the risk of coronary atherosclerosis in men. In addition, Ginsberg *et al* (247) found that myocardial ischemia was associated with elevated postprandial plasma triglyceride concentrations independent of other risk factors including fasting LDL and HDL concentrations. This is confirmed by more recent studies that document plasma removal of chylomicron remnants is delayed in normolipidemic male patients with CHD (248) and also in normolipidemic women with CHD (249,250). Furthermore,

women in menopause with or without CHD have slower removal of chylomicron remnants and this raises the possibility that a deficiency of estrogen may be associated with impaired postprandial metabolism (251). These observations strongly indicate that slow removal of chylomicron remnants is an independent risk factor for atherosclerosis.

In other dsylipidemic conditions, patients with homozygous or heterozygous familial hypercholesterolemia have slower removal of chylomicron remnants (200,252), however, Rubinstein *et al* (107) and Eriksson *et al* (253) found no such impairment. This disparity in observations may be due to different methodologies used. Since there are few or no LDL receptors in patients with familial hypercholesterolemia, these differing observations raise the question of whether non-LDL receptor pathways are up-regulated in such cases. Slower plasma remnant removal has also been observed in patients with familial combined hyperlipidemia (FCH), a disease characterized by disturbances of triglyceride metabolism and premature atherosclerosis (76,254).

Non-insulin-dependent-diabetes-mellitus NIDDM) is also strongly associated with hypertriglyceridemia and impaired postprandial lipoprotein metabolism is a common disorder in patients with this condition (255,256,257,258,259,260,261). Of interest as well is the dyslipidemic condition known as syndrome X which is characterized by a combination of clinical symptoms that include hypertriglyceridemia, insulin resistance, glucose intolerance, hypertension and obesity (262,263,264). Male or female patients who have a combination of these disorders also have impaired plasma removal of remnant lipoproteins and they are prone to premature atherosclerosis (265,266,267).

Since the LDL receptor is an important receptor, remnant removal is likely to be affected by the number of receptors. The expression of LDL receptor numbers on chylomicron remnant removal was examined in two studies by controlling the cholesterol in the diet. Ginsberg *et al* (176) found that cholesterol-enriched diets had no effect on plasma remnant removal in young normolipidemic individuals. In the other study, Clifton and Nestel (177) came to the conclusion that cholesterol feeding had no effect on plasma remnant removal in normolipidemic men but interestingly, there was decreased removal in hypertriglyceridemic men. The fat feeding would, in theory, have down-regulated LDL receptors, however, the results are consistent with the hypothesis that there are non-LDL receptor pathways that become significant only if the LDL receptor pathway is partially or fully saturated (107,253).

8. HOW CHYLOMICRON REMNANTS MAY CAUSE ATHEROGENESIS

Russell Ross (178,179,268) has suggested that atherosclerosis occurs in response to inflammation in the arterial wall probably initiated by toxic substances. Some of these toxic substances may include oxidized LDL, oxysterols and oxidized protein products (269,270). By using selected-affinity anti-apoB immunosorption, Rapp *et al* (201) found that human atherosclerotic plaques contain intact remnants of triglyceride-rich lipoproteins, which strongly suggest that remnants may be one of these lipoproteins.

One of the most difficult questions to prove is whether other lipoproteins, besides LDL, cause and/or participate in the development of atherosclerosis. Many factors such as beta-VLDL, lipoprotein (a), homocysteine and viruses (271,272,273,274,275,276) have been implicated in the pathogenesis of this complex disease. The amounts of different lipoproteins, however, may vary in separate lesions. LDL may be in higher amounts than chylomicron remnants in some lesions and vice-versa in other lesions. The difficult question to answer is whether the lipoprotein that is greatest in amount in a particular lesion is the major participant in triggering the cellular responses such as attraction of monocytes to the subendothelial space, stimulation of smooth muscle growth, foam cell formation that lead to atherosclerosis, or whether small quantities of potentially atherogenic particles initiate the lesion and the other lipoproteins then participate.

The cellular responses that chylomicron remnants potentially initiate to promote and accelerate atherosclerosis development are not clearly defined; however, based on current knowledge, several possibilities exist. The earliest identifiable event in the development of atherosclerosis is the adhesion of monocytes to endothelial cells (179,268). This requires the change in the level of expression of a number of already identified and perhaps some unknown factors on both of the cell types (202,203,207,209). It has been found that atherogenic lipoproteins such as oxidized and minimally modified LDL can initiate this event (208,210). In addition, monocytes from patients with diabetes, a condition where there is delayed remnant removal, are adherent to cultured endothelial cells (277,278). Our laboratory has recently found that remnant-rich fractions of serum can modulate this event (Wilhelm, Fong and Cooper, unpublished observations). Thus, it is possible that either or both remnant concentration or compounds in the remnants can initiate the earliest event of atherosclerosis. Obviously, this is likely to be a transient and reversible phenomenon but identification of the properties of these physiologically relevant lipids or proteins that transduce this phenonmenon should be interesting and potentially important.

In addition to the effects in the circulation, there is transcytosis of chylomicron remnants across the endothelium and into the subendothelial and medial layers (see figure 3). The extent of remnant transcytosis could be dependent on the duration and magnitude of the postprandial period. In the arterial wall, remnants are degraded efficiently by smooth muscle cells, thus eliminating retention and accumulation; however, when there is down-regulation of LDL receptors, there will be increased retention and accumulation of remnants in the extracellular space of the subendothelial and medial layers. In the intima, the monocytes differentiate into macrophages

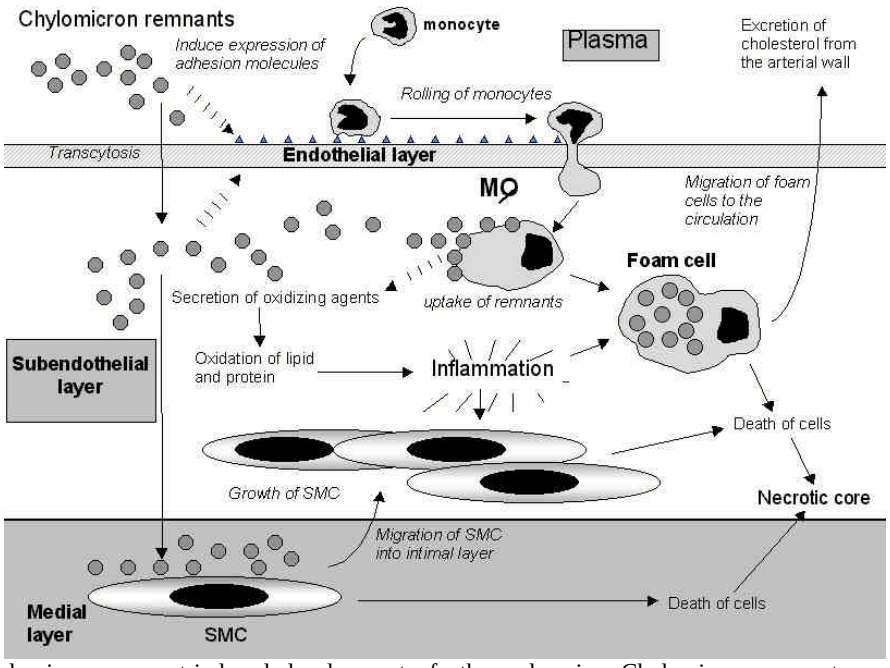

Figure 3. Chylomicron remnant-induced development of atherosclerosis. Chylomicron remnants may induce changes in endothelial cells and perhaps monocytes that cause monocyte rolling followed by adhesion. Chylomicron remnants cross from the plasma compartment into the subendothelial layer by transcytosis. The remnants continue to migrate into the medial layer. In the normal situation, smooth muscle cells in the medial layer are able to remove remnants efficiently. When there is excessive amounts of remnants in the arterial wall, smooth muscle cells are unable to compensate. This forms a barrier to the continual flux of remnants migrating from the subendothelial layer into the medial layer. The retention and accumulation of remnants in the subendothelial layer may induce expression of adhesion molecules on the endothelial surface. The expression of endothelial surface adhesion molecules may be exacerbated by remnants in the plasma compartment if there is prolonged residence of these lipoproteins in the blood circulation. Blood monocytes bind to the adhesion molecules; this leads to rolling of the monocytes along the endothelial layer, followed by migration of the monocytes into the subendothelial layer where the monocytes differentiate into macrophages. The macrophages take up remnants and begin to become foam cells. The foam cells are able to eliminate the remnants by migrating back into the plasma compartment. If remnant uptake is excessive, there may be death of foam cells. In addition, the macrophages may be induced to secrete toxic oxidizing agents (superoxide, free radicals, etc.) which may cause oxidation of lipid and protein, hence causing inflammation. The oxidized products may cause death of foam cells. At the same time, smooth muscle cells may migrate from the medial layer to the subendothelial layer and under the influence of mitogenic factors secreted by macrophages and/or foam cells, they may start multiplying (growth). The increased number of smooth muscle cells will cause deposition of fibrous tissue and producing raised plaques which protrude into the blood lumen. Remnants and/or the oxidized products may cause death of the smooth muscle cells. A necrotic core will be produced by the death of cells. Abbreviations: MØ, macrophage; SMC, smooth muscle cells.

and take up retained remnants. There is evidence that suggest lipid-laden macrophages can leave the arterial wall and return back to the circulation (279). In such a pathway, the excessive chylomicron remnants can be removed efficiently from the extracellular space. In some situations, the macrophages may remain in the arterial wall, continue to take up chylomicron remnants, and ultimately become foam cells (see figure 3). This may well explain the formation of fatty streaks that can be transitory in nature. In the formation of advanced lesions, toxic substances (oxidizing agents, cytokines) may be secreted by macrophages and they may harm the arterial wall. For example, superoxide free radicals may oxidize LDL and other lipoproteins and lipids to produce far more toxic oxidized products. These products potentially cause damage to the arterial wall, thus producing inflammatory responses. Furthermore, growth of smooth muscle cells occurs induced by macrophage-secreted cytokines and this leads to formation of the fibrous cap. In far more advanced lesions, there will be death of cells and this produces necrotic cores. Cell death may be caused by chylomicron remnants as it has been demonstrated that these lipoproteins can cause death of smooth muscle cells and macrophages *in vitro* (227,280,281). Also, evidence of remnant cytotoxicity was provided previously by Chung and co-workers (282) who found that remnants of lipolyzed triglyceride-rich lipoproteins from hypertriglyceridemic human serum were cytotoxic to macrophages because of the high concentration of free fatty acids produced after lipolysis. In addition, remnants may also be involved in the coagulation pathway. Remnants of triglyceride-rich lipoproteins have been shown to promote activation of factor VII, an enzyme that is important in the production of thrombin, an enzyme that converts fibrinogen to fibrin in the development of a clot (283,284,285). It appears that the free fatty acids produced by lipolysis of triglyceride-rich

lipoproteins at the interface of circulating remnants and the endothelium provide a contact surface that activates the contact system of coagulation and subsequently activates factor VII (283). This is particularly damaging at atherosclerotic plaques. The fibrin clot will trap platelets which may exacerbate injury to the plaques.

This proposed model (figure 3) is not an attempt to suggest that chylomicron remnants are the only atherogenic lipoprotein. There is no doubt that other lipoproteins (LDL, beta-VLDL, lipoprotein (a)), are also atherogenic. Remnants may act in tandem with other lipoproteins to produce the atherogenic effects mentioned above.

9. PERSPECTIVE

Atherosclerosis remains a major health ailment in the United States and in other western societies (286,287). Although LDL is now established as a 'bad cholesterol', there has always been skepticism about the atherogenicity of chylomicron remnants. The studies presented in this review are only a sample of an increasing number of studies that implicate remnants as a 'culprit' in atherosclerosis. In summary, the main thrusts of this review have been to emphasize the importance of an efficient removal system for chylomicron remnants and that chylomicron remnants may be directly causing atherogenesis. The chylomicron remnant removal system is a combination of different pathways dependent and/or independent of each other. Perhaps such a complex system for dealing with dietary-derived lipid has evolved because of the serious consequence of a poor ability to remove remnants from the blood circulation. The average human perhaps spend a great part of their life in the postprandial state which represents a constant repeated insult to the arterial wall. We are now beginning to understand the complicated metabolism of dietary-derived lipid in the body and should be able to use this knowledge in clinical testing for the prevention of CHD.

10. ACKNOWLEDGEMENTS

This work was supported in part by a National Institutes of Health grant (DK38218), a Stanford University Digestive Disease Center grant (DK38707), and an American Heart Association (Western States Affiliate) postdoctoral fellowship (9920129Y) (awarded to K.C-W. Yu). We thank Mr. Rick Cuevas for preparation of the manuscript.

11. REFERENCES

1. Zilversmit D.B.: Atherogenesis: a postprandial phenomenon. *Circulation* 60, 473-485 (1979)

2. Havel, R.J.: Postprandial hyperlipidemia and remnant lipoproteins. *Curr Opin Lipidol* 5, 102-119 (1994)

3. Karpe, F. & A. Hamsten: Postprandial lipoprotein metabolism and atherosclerosis. *Curr Opin Lipidol* 6, 123-129 (1995)

4. Ebenbichler, C.F., R. Kirchmair, C. Egger, C. & J.R. Patsch: Postprandial state and atherosclerosis. *Curr Opin Lipidol* 6, 286-290 (1995)

5. Cooper, A.D.: Hepatic uptake of chylomicron remnants. *J Lipid Res* 38, 2173-2192 (1997)

6. Havel, R.J.: Receptor and non-receptor mediated uptake of chylomicron remnants by the liver. *Atherosclerosis* 141 (supplement 1), S1-S7 (1998)

7. Mahley, R.W. & Z.S. Ji.: Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J Lipid Res* 40, 1-16 (1999)

8. Brown, M.S. & J.L. Goldstein: A receptor-mediated pathway for cholesterol homeostasis. *Science* 232, 34-47 (1986)

9. Redgrave, T.R.: Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J Clin Invest* 49, 465-471 (1970)

10. Glickman, R.M., K. Kirsch & K.J. Isselbacher: Fat absorption during inhibition of protein synthesis: studies of lymph chylomicrons. *J Clin Invest* 51, 356-363 (1972)

11. Glickman, R.M. & K. Kirsch: Lymph chylomicron formation during the inhibition of protein synthesis. Studies of chylomicron apoproteins. *J Clin Invest* 52, 2910-2120 (1973)

12. Imaizumi, K., M. Fainaru & R.J. Havel: Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J Lipid Res* 19, 712-722 (1978)

13. Mahley, R.W., T.L. Innerarity, S.C. Rall & K.H. Weisgraber: Plasma lipoproteins: apolipoprotein structure and function. *J Lipid Res* 25, 1277-1285 (1984)

14. Ginsberg, H.N.: Lipoprotein metabolism and its relationship to atherosclerosis. *Med Clin North Am* 78, 1-20 (1994)

15. Brown, M.S., P.T. Kovanen & J.L. Goldstein: Regulation of plasma cholesterol by lipoprotein receptors. *Science* 628-635 (1981)

16. Havel, R.J.: Lipid transport function of lipoproteins in blood plasma. *Am J Physiol* 253, E1-E5 (1987)

17. Powell, C.M., S.C. Wallis, R.J. Pease, Y.H. Edwards, T.J. Knott & J. Scott: A novel form of tissue-specific RNA processing produces apolipoprotein B48 in intestine. *Cell* 50, 831-840 (1987)

18. Tennyson, G.E., C.A. Sabatos, K. Higuchi, N. Meglin & A. Brewer, Jr.: ApoB-48 produced by liver and intestine. *Proc Natl Acad Sci USA* 86, 500-505 (1989)

19. Ockner, R.K., F.B. Hughes & K.J. Isselbacher: Very low density lipoproteins in intestinal lymph: origin, composition, and role in lipid transport in the fasting state. *J Clin Invest* 48, 2079-2088 (1969)

20. Martins, I.J., A.J. Sainbury, J.C. Mamo & T.G. Redgrave: Lipid and apolipoprotein B-48 transport in mesenteric lymph and the effect of hyperphagia on the clearance of chylomicron-like emulsions in insulin-deficient rats. *Diabetologia* 37, 238-246 (1994)

21. Farese, Jr., R.V., M.M. Veniant, C.M. Cham, L.M. Flynn, V. Pierotti, J.F. Loring, M. Traber, S. Ruland, R.S. Stokowski, D. Huszar & S.G. Young: Phenotypic analysis of mice expressing exclusively apolipoprotein B48 or apolipoprotein B100. *Proc Natl Acad Sci USA* 93, 6393-6398 (1996)

22. Veniant, M.M., V. Pierotti, D. Newland, C.M. Cham, D.A. Sanan, R.L. Walzem & S.G. Young: Susceptibility to atheroscelerosis in mice expressing exclusively apolipoprotein B48 or apolipoprotein B100. *J Clin Invest* 100, (1997)

23. Hamilton, R.J., J.S. Wong, C.M. Cham, L.B. Nielsen & S.G. Young: Chylomicron-sized lipid particles are formed in the setting of apolipoprotein B deficiency. *J Lipid Res* 39, 1543-1557 (1998)

24. Jung, H.R., S.M. Turner, R.A. Neese, S.G. Young & M.K. Hellerstein: Metabolic adaptations to dietary fat malabsorption in chylomicron-deficient mice. *Biochem J* 343, 473-478 (1999)

25. Korn, E.D.: Clearing factor, a heparain activated lipoprotein lipase. *J Biol Chem* 215, 1-5 (1955)

26. Mjos, O.D., O. Faegerman, R.L. Hamilton & R.J. Havel.: Characterization of remnants produced during the metabolism of triglyceride-rich lipoprotein of blood plasma and intestinal lymph in the rat. *J Clin Invest* 56, 603-615 (1975)

27. Eisenberg, S., E. Sehayek, T. Olivecrona & I. Vlodavsky: Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate cell-surface and extracellular matrix. *J Clin Invest* 90, 2013-2021 (1992)

28. Olivecrona, T. & G. Bengtsson-Olivecrona: Lipoprotein lipase and hepatic lipase. *Curr Opin Lipidol* 1, 222-230 (1990)

29. Olivecrona, T. & G. Bengtsson-Olivecrona: Lipoprotein lipase and hepatic lipase. *Curr Opin Lipidol* 4, 187-196 (1993)

30. Havel, R.J.: Role of the liver in atherosclerosis. *Arteriosclerosis* 5, 569-580 (1985)

31. Tall, A.R. & D.M. Small: Plasma high-density lipoproteins. *New Engl J Med* 299, 123201236 (1978)

32. Redgrave, T.G. & D.M. Small: Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoproein fraction during the catabolism of chylomicrons in the rat. *J Clin Invest* 64, 162-167 (1979)

33. Havel, R.J.: Lipoprotein biosynthesis and metabolism. *Ann NY Acad Sci* 348, 16-29 (1980)

34. Choi, S.Y. & A.D. Cooper: A comparison of the roles of the LDL receptor and the LDL receptor-related protein/alpha 2-macroglobulin receptor in chylomicron remnant removal in the mouse in vivo. *J Biol Chem* 268, 15804-15811 (1991)

35. Hay, R. & G.S. Getz: Translation *in vivo* and *in vitro* of proteins resembling apoproteins of rat plasma very low density lipoproteins. *J Lipid Res* 20, 334-348 (1979)

36. Assmann, G.: Lipid metabolism and atherosclerosis. Schattauer-Velag. (1982)

37. Glomset, J.A.: The plasma Lecithin:cholesterol acyltransferase reaction. *J Lipid Res* 9, 155-167 (1968)

38. Schmitz, G. & E. Williamson: High density lipoprotein metabolism, reverse cholesterol transport and membrane protection. *Curr Opin Lipidol* 2, 177-189 (1991)

39. Goldstein, J.L. & M.S. Brown: The low-density-lipoprotein pathway and its relation to atherosclerosis. *Ann Rev Biochem* 46, 897-930 (1977)

40. Gordon, T., W.B. Kannel, W.P. Castelli & T.R. Dawber: Lipoproteins, cardiovascular diseases and death: the Framingham Study. *Arch Int Me*d 141, 1128-1131 (1981)

41. Miller, G.J: High density lipoproteins and atherosclerosis. *Ann Rev Med* 31, 97-108 (1980)

42. Pattnaik, N.M., A. Montes, L.B. Hughes & D.B. Zilversmit: Cholesteryl ester exchange protein in human plasma isolation and characterization. *Biochim Biophys Acta* 530, (1978)

43. Chajek, T. & C.J. Fielding: Isolation and characterization of a human serum cholesteryl ester transfer protein. *Proc Natl Acad Sci USA* 75, (1978)

44. Fielding, P.E. amd C.J. Fielding: A cholesteryl ester transfer complex in human plasma. *Proc Natl Acad Sci USA* 77, (1980)

45. Fainaru, M., R.W. Mahley, R.L. Hamilton & T.L. Innerarity: Structural and metabolic heterogenicity of beta-VLDL from cholesterol-fed dogs and from humans with type III hyperlipoproteinemia. *J Lipid Res* 23, 702-714 (1982)

46. Mahley, R.W.: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622-630 (1988)

47. Mahley, R.W., Y. Huang & S.C. Rall, Jr.: Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia) Questions, quandaries and paradoxes. *J Lipid Res* 40, 1933-1949 (1999)

48. Goldstein, J.L., Y.K. Ho, M.S. Brown, T.L. Innerarity & R.W. Mahley: Cholesteryl ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic beta-very-low-density-lipoproteins. *J Biol Chem* 255, 1839-1848 (1980)

49. van Lenten, B.J., A.M. Fogelman, M.M. Hokom, L. Benson, M.E. Haberland & P.A. Edwards: Regulaion of the uptake and degradation of beta-very-low density lipoprotein in human monocyte macrophages. *J Biol Chem* 258, 5151-5157 (1983)

50. Koo, C., M.E. Wernette-Hammond & T.L. Innerarity: Uptake of canine beta-very-low-density-lipoproteins by mouse peritoneal macrophages is mediated by a low density lipoprotein receptor. *J Biol Chem* 261, 11194-11201 (1986)

51. Tabas, I., S. Lim, S., X.X. Xu & F.R. Maxfield: Endocytosed beta-VLDL and LDL are delivered to different intracellular vesicles in mouse peritoneal macrophages. *J Cell Biol* 111, 929-940 (1990)

52. Xu, X.X. & I. Tabas: Lipoproteins activate acyl-coenzyme A: cholesterol acyltransferase in macrophages only when cellular cholesterol pools are expanded to a critical threshold level. *J Biol Chem* 266, 17040-17048 (1991)

53. Moreton, J.R.: Chylomicronemia, fat tolerance and atherosclerosis. *J Lab Clin Med* 35, 373-384 (1950)

54. Woldow, A., A.J.E. Chapman & J.M. Evans: Fat tolerance in subjects with atherosclerosis, heparin effects upon lipemia, lipoprotein and gamma globulin. *Am Heart J* 47, 568-579 (1954)

55. Brown, D.F., A.S. Heslin & J.T. Doyle: Postprandial lipemia in health and in ischaemic heart disease. A comparison of three indexes of fat absorption and removal and their modification by systemic heparin adminstration. *New Engl J Med* 264, 733-737 (1961)

56 Kirchmair, R., C.F. Ebenbichler & J.R. Patsch: Postprandial lipemia. *Baill Clin Endocrinol Met* 9, 705-719 (1995)

57. Quarfordt, S.H. & D.S. Goodman: Incorporation of labeled cholesteryl esters into chylomicrons in vitro. *J Lipid Res* 7, 708-710 (1966)

58. Quarfordt, S.H. & D.S. Goodman: Metabolism of double-labeled chylomicron cholesteryl esters in the rat. *J Lipid Res* 8, 264-273 (1967)

59. Redgrave, T.G., K.B. Dunne, D.C.K. Roberts & C.E. West: Chylomicron metabolism in rabbits fed diets with or without added cholesterol. *Atherosclerosis* 24, 501-508 (1976)

60. Redgrave, T.G. & G. Martin: Effects of chronic ethanol consumption on the catabolism of chylomicron triacylglycerol and cholesterol ester in the rat. *Atherosclerosis* 28, 69-80 (1988)

61. Sherril, B.C., T.L. Innerarity & R.W. Mahley: Rapid hepatic clearance of canine lipoproteins containing only the E apoprotein by a high affinity receptor. Identity with the chylomicron remnant transport process. *J Biol Chem* 255, (1980)

62. Windler, E., Y. Chao & R.J. Havel: Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnatns in the rat. *J Biol Chem* 255, 5475-5480 (1980)

63. Kita, T., J.L. Goldstein, M.S. Brown, Y. Watanabe, C.A. Hornick & R.J. Havel: Hepatic uptake of chylomicron remnants in WHHL rabbits: a mechanism genetically distinct from the low density lipoprotein receptor. *Proc Natl Acad Sci USA* 79, 3623-3627 (1982)

64. Redgrave, T.G. & R.C. Maranhao: Metabolism of protein-free lipid emulsion models of chylomicrons in rats. *Biochim Biophys Acta* 835, 104-112 (1985)

65. Redgrave, T.G., D.R. Kodali & D.M. Small: The effect of triacyl-sn-glycerol structure on the metabolism of chylomicrons and triacylglycerol-rich emulsions in the rat. *J Biol Chem* 263, 5118-5123 (1988)

66. Mortimer, B.C., W.J. Simmonds, C.A. Joll, R.V. Stick & T.G. Redgrave: Regulation of the metabolism of lipid emulsion model lipoproteins by a saturated acyl chain at the 2-position of triacylglycerol. *J Lipid Res* 29, 713-720 (1988)

67. Mortimer, B.C., D.J. Beveridge, I.J. Martins & T.G. Redgrave: Intracellular localization and metabolism of chylomicrons remnants in the livers of low density lipoprotein receptor-deficient mice and apoE-deficient mice. Evidence for slow metabolism via an alternative apoE-dependent pathway. *J Biol Chem* 270, 28767-28776 (1995)

68. Ishibashi, S., J. Herz, N. Meada, J.L. Goldstein & M.S. Brown: The two-receptor model of lipoprotein clearance: tests of the hypothesis in "knockout" mice lacking the low density lipoprotein receptor, apolipoprotein E, or both proteins. *Proc Natl Acad Sci USA* 91, 4431-4435 (1994)

69. Redgrave, T.G. & D.A. Snibson: Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Metabolism* 26, 493-503 (1977)

70. Mokuno, H., S. Brady, L. Kotite, J. Herz & R.J. Havel: Effect of the 39-kDa receptor-associated protein on the hepatic uptake and endocytosis of chylomicron remnants

and low density lipoproteins in the rat. *J Biol Chem* 269, 13238-13243 (1994)

71. Redgrave, T.G., I.J. Martins & B-C. Mortimer: Measurement of expired carbon dioxide to access the metabolism of remnant lipoproteins. *J Lipid Res* 36, 2670-2675 (1995)

72. Martins, I.J. & T.G. Redgrave: A 13CO2 breath test to assess the metabolism of triglyceride-rich lipoprotein remnants in mice. *J Lipid Res* 39, 691-698 (1998)

73. Karpe, F., M. Bell, J. Bjorkegren, J. & A. Hamsten: Quantification of postprandial triglyceride-rich lipoproteins in healthy men by retinyl ester labeling and simultaneous measurement of apolipoprotein B-48 and B-100. *Arter Thromb Vasc Biol* 15, 199-207 (1995)

74. Kransinski, S.D., J.S. Cohn, R.M. Russel & E.J. Schaefer: Postprandial plasma vitamin A metabolism in humans: a reassessment of the use of plasma retinyl esters as markers for intestinally derived chylomicrons and their remnants. *Metabolism* 4, 357-365 (1990)

75. Patsch, J.R., G. Miesenbock, T. Hopferwieser, V. Muhlberger, E. Knapp, E., J. Day Dunn, A.M. Gotto, Jr. & W. Patsch: Relation of triglyceride metabolism and coronary artery disease. *Ater Thromb* 12, 1336-1345 (1992)

76. Castro-Cabezas, M., D.W. Erkelens, L.A.W. Kock & T.W.A. de Bruin: Postprandial apolipoprotein B-100 and B-48 metabolism in familial combined hyperlipidemia before and after reduction of fasting plasma triglycerides. *Eur J Clin Invest* 24, 252-257 (1994)

77. Poapst, M., K. Uffelman & G. Steiner: The chromogenicity and quantitation of apoB-100 and apoB-48 of human plasma lipoproteins on analytical SDS gel electrophoresis. *Atherosclerosis* 65, 75-88 (1987)

78. Karpe, F. & A. Hamsten: Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. *J Lipid Res* 35, (1994)

79. Mamo, J.C.L., D. Smith, K.C-W. Yu, A. Kawaguchi, M. Harada-Shiba, T. Yamamura & A. Yamamoto: Accumulation of chylomicron remnants in homozygous subjects with familial hypercholesterolemia. *Eur J Clin Invest* 28, 379-384 (1998)

80. Peel, A.S., A. Zampelas, C.M. Williams & B.J. Gould: A novel antiserum specific to apolipoprotein B-48 in the investigation of postprandial lipidemia in humans. *Clin Sci* 85, 521-524 (1993)

81. Uchida, Y., Y. Kurano & S. Ito: Establishment of monoclonal antibody against human apoB-48 and measurement of apoB-48 in serum by ELISA method. *J Clin Lab Anal* 12, 289-292 (1998)

82. Hazzard, W.R. & E.L. Bierman: Delayed clearance of chylomicron remnants following vitamin-A-containing oral fat loads in broad-beta-disease (type III hyperlipoproteinemia) *Metabolism* 25, 777-801 (1976)

83. Berr, F. & F. Kern, Jr.: Plasma clearance of chylomicrons labeled with retinyl palmitate in healthy human subjects. *J Lipid Res* 25, 805-812 (1984)

84. Kransinski, S.D., J.S. Cohn, E.J. Schaefer & R.M. Russell: Postprandial plasma retinyl ester responses is greater in older subjects compared with younger subjects: evidence for delayed plasma clearance of intestinal lipoproteins. *J Clin Invest* 85, 883-892 (1990)

85. Cohn, J.S., E.J. Johnson, J.S. Miller, S.D. Cohn, R.W. Milne, Y.L. Marcel, R.M. Russell & E.J. Schaefer: Contribution of apoB48 and apoB100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TR triglycerides and retinyl esters. *J Lipid Res* 34, 2033-2040 (1993)

86. Schaefer, J.R., D.J. Rader & H.B. Brewer, Jr: Investigation of lipoprotein kinetics using endogenous labeling with stable isotopes. *Curr Opin Lipidol* 3, 227-232 (1992)

87. Watson, T.D.G.: New in-vivo techniques for measuring lipoprotein metabolism. *Curr Opin Lipidol* 6, 182-186 (1995)

88. Ikewaki, K., D.J. Rader, J.R. Schaefer, T. Fairwell & L.A. Zech: Evaluation of apoA-1 kinetics in humans using simultaneous endogenous stable isotope and exogenous radiotracer methods. *J Lipid Res* 34, 2207-2215 (1993)

89. Dermant, T.H., C.J. Packard, P. Steward, A. Bedynek, A.G. Calder, J. Shepherd & D. Seidel: Sensitive mass spectrometry techniques for measuring metabolism of human apolipoprotein B in vivo. *Clin Chem* 40, 1825-1827 (1994)

90. Foster, D.M., P.H.R. Barrett, G. Toffolo, W.F. Beltz & C. Ocello: Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data. *J Lipid Res* 34, 2193-2205 (1993)

91. Faix, D., R. Neese, C. Ikletke, S. Wolden, D. Cesar & M. Coutlangus: Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans. *J Lipid Res* 33, 2063-2075 (1993)

92. Welty, F.K., A.H. Lichtenstein, P.H. Barrett, G.G. Dolnikowski & E.J. Schaefer: Human apolipoprotein B-48 and apoB-100 kinetics with stable isotopes. *Arter Thromb Vasc Biol* 19, 2966-2974 (1999)

93. Miensenbock, G. & J.R. Patsch: Postprandial hyperlipidemia: the search for the atherogenic lipoprotein. *Curr Opin Lipidol* 3, 196-201 (1992)

94. Junqueira, L.C., J. Carneiro & R.O. Kelley: Basic Histology. Appleton and Lange. (1989)

95. Wisse, E., R.B. de Zanger, P. van der Smissen & R.S. McCuskey: The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of disse. *Hepatology* 5, 683-692 (1985)

96. Fraser, R., B.R. Dobbs & G.W.T. Rogers: Lipoproteins and the liver sieve:the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis and cirrhosis. *Hepatology* 21, 863-874 (1995)

97. Windler, E., J. Greeve, H. Robenek, F. Rinninger, H. Greten & S. Jackle: Differences in the mechanisms of uptake and endocytosis of small and large chylomicron remnants by rat liver. *Hepatology* 24, 344-351 (1996)

98. Wisse, E. & D.L. Knook: Kupffer cells and other sinusoidal cells. Elservier. (1977)

99. Hendricks, H.F.J., W.A. Verhostaad, A. Brouwer, A.M. de Leeuw & D.L. Knook: Perisinusoidal fat storing cells are the main vitamin A storage sites in rat liver. *Exp Cell Res* 160, 138-149 (1985)

100. Bouwens, L., P. de Bleser, K. Vanderkerken, B. Geerts & E. Wisse: Liver cell heterogeneity: functions of non-parenchymal cells. *Enzyme* 46, 155-168 (1992)

101. Mahley, R.W. & M.M. Hussain: Chylomicron and chylomicron remnant catabolism. *Curr Opin Lipidol* 2, 170-176 (1991)

102. Beisiegel, U.: Receptors for triglyceride-rich lipoproteins and their role in lipoprotein metabolism. *Curr Opin Lipidol* 6, 117-122 (1995)

103. Innerarity, T.L. & R.W. Mahley: Enhanced binding of cultured human fibroblasts of apoE-containing lipoproteins as compared with low density lipoproteins. *Biochemistry* 17, 1440-1446 (1978)

104. Weisgraber, K.H., T.L. Innerarity & R.W. Mahley: Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. *J Biol Chem* 257, 2518-2521 (1982)

105. Mahley, R.W. and Y.D. Huang: Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Curr Opin Lipidol* 10, 207-217 (1999)

106. Pitas, R.E., T.L. Innerarity & R.W. Mahley: Cell surface receptor binding of phospholipid-protein complexes containing different ratios of receptor-active and -inactive E apoproteins. *J Biol Chem* 255, 5454-5460 (1980)

107. Rubinstein, D.C., J.C. Cohen, M.G. Berger, D.R. van der Westhuyzen & G.A. Coetzee: Chylomicron remnant clearance from the plasma is normal in familial hypercholesterolemic homozygotes with defined receptor defects. *J Clin Invest* 86, 1306-1312 (1990)

108. Nagata, Y., J. Chen & A.D. Cooper: Role of low density lipoprotein-dependent and -independent sites in binding and uptake of chylomicron remnants in rat liver. *J Biol Chem* 263, 15151-15158 (1988)

109. Choi, S.Y., L.F. Fong, M.J. Kirven & A.D. Cooper: Use of an anti-LDL receptor antibody to quantify the role of the LDL receptor in the removal of chylomicron remnants in the mouse in vivo. *J Clin Invest* 88, 1173-1181 (1991)

110. Beaumont, J.L. & F. Assadollahi: Retinyl palmitate labeled intestinally derived lipoproteins accumulate in the circulation of WHHL rabbits. *Atherosclerosis* 85, 103-111 (1990)

111. Bowler, A., T.G. Redgrave & J.C.L. Mamo: Chylomicron remnant clearance in homozygote and heterozygote Watanabe-heritable-hyperlipidaemic rabbits is defective. *Biochem. J.* 276, 381-386 (1991)

112. Hussain, M.M., T.L. Innerarity, W.J. Brecht & R.W. Mahley: Chylomicron metabolism in normal, cholesterol-fed and Watanabe heritable hyprlipidemic rabbits. Saturation of the sequestration step of the remnant clearance pathway. *J Biol Chem* 270, 8578-8587 (1995)

113. de Faria, E., L.F. Fong, M. Komaromy & A.D. Cooper: Relative roles of the LDL receptor, the LDL receptor-like protein and hepatic lipase in chylomicron remnant removal by the liver. *J Lipid Res* 37, 197-209 (1996)

114. Yu, K.C-W., Y. Jiang, W. Chen & A.D. Cooper: Evaluation of the components of the chylomicron remnant removal mechanism by use of the isolated perfused mouse liver. *J Lipid Res* 40, 1899-1910 (1999)

115. Herz, J., S.Q. Qiu, A. Oesterle, H.V. DeSilva, S. Shafi & R.J. Havel: Initial hepatic removal of chylomicron remnants is unaffected but endocytosis is delayed in mice lacking the low density lipoprotein receptor. *Proc Natl Acad Sci USA* 92, 4611-4615 (1995)

116. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl & K.K. Stanley: Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL receptor suggest a physiological role as lipoprotein receptor. *EMBO J* 7, 4119-4127 (1988)

117. Beisiegel, U., W. Weber, G. Ihrke, J. Herz & K.K. Stanley: The LDL receptor-related protein, LRP, is an apolipoproein E-binding protein. *Nature* 341, 162-164 (1989)

118. Chappell, D.A., G.L. Fry, M.A. Waknitz, P.H. Iverius, S.E. Williams & D.K. Strickland: The low density lipoprotein receptor-related protein/alpha 2-macroglobulin

receptor binds and mediates catabolism of bovine milk lipoprotein lipase. *J Biol Chem* 267, 25764-25767 (1992)

119. Willnow, T.E., Z. Sheng, S. Ishibashi & J. Herz: Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science* 264, 1471-1474 (1994)

120. Krieger, M. & J. Herz: Structures and functions of multiligand lipoprotein receptors: macrophages scavenger receptors and LDL receptor-related protein (LRP) *Ann Rev Biochem* 63, 601-637 (1994)

121. Brown, M.S. & J.L. Goldstein: Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Ann Rev Biochem* 52, 223-261 (1983)

122. Kowal, R.C., J. Herz, J.L. Goldstein, V. Esser & M.S. Brown: Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc Natl Acad Sci USA* (1989)

123. Strickland, D.K., J.D. Ashcom, S. Williams, W.H. Burgess, M. Migliorini & W.S. Argraves: Sequence identity between the alpha 2-macroglobulin receptor and LDL receptor-related protein suggests that this molecule is a multifunctional receptor. *J Biol Chem* 265, 17401-17404 (1990)

124. Bu, G., E.A. Maksymovitch, H. Geuze & A.L. Schwartz: Subcellular localization and endocytic function of low density lipoprotein-related protein in human glioblastoma cells. *J Biol Chem* 269, 29874-29882 (1994)

125. Bu, G., H.J. Geuze, G.J. Strous & A.L. Schwartz: 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein. *EMBO Journal* 14, 2269-2280 (1995)

126. Bu, G. and S. Rennke: Receptor-associated protein is a folding chaperone for low density lipoprotein receptor-related protein. *J Biol Chem* 271, 22218-22224 (1996)

127. Bu, G.: Receptor-associated protein: a specialized chaperone and antagonist for members of the LDL receptor gene family. *Current Opinion of Lipidology* 9, 149-155 (1998)

128. Herz, J., J.L. Goldstein, D.K. Strickland, Y.K. Ho & M.S. Brown: 39-kDa protein modulates binding of ligands to low density lipoprotein-related-protein/alpha 2-macroglobulin receptor. *J Biol Chem* 266, 21232-21238 (1991)

129. Moestrup, S.K. & J. Gliemann: Analysis of ligand recognition by the purfied alpha 2-macroglobulin receptor(LRP) Evidence that high affinity of alpha 2-macroglobulin-proteinase complex is acheived by binding to adjacent receptors. *J Biol Chem* 266, 14011-14017 (1991)

130. Herz, J. & T.E. Willnow: Gene transfer and disruption strategies to elucidate hepatic lipoprotein receptor function. *Atherosclerosis* 118 (suppl), S37-S41 (1995)

131. Rohlmann, A., R. Gotthardt, R.E. Hammer & J. Herz: Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms the role of LRP in clearance of chylomicron remnants. *J Clin Invest* 101, 689-695 (1998)

132. Stow, J.L., L. Kjellen, E. Unger, M. Hook & M.G. Farquhar: Heparan sulfate proteoglycans are concentrated on the sinusoidal plasmalemmal domain and in intracellular organelles of hepatocytes. *J Cell Biol* 100, 975-980 (1985)

133. Hamilton, R.L., J.S. Wong, L.S. Guo, S. Krisans & R.J. Havel: Apolipoprotein E localization in rat hepatocytes by immungold labeling of cryothin sections. *J Lipid Res* 31, 1589-1603 (1990)

134. Ji, Z.S., W.J. Brecht, R.D. Miranda, M.M. Hussain, T.L. Innerarity & R.W. Mahley: Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *J Biol Chem* 268, 10160-10167 (1993)

135. Ji, Z.S., S. Fazio, Y.L. Lee & R.W. Mahley: Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. *J Biol Chem* 269, 2764-2772 (1994)

136. Ji, Z.S., D.A. Sanan & R.W. Mahley: Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. *J Lipid Res* 36, 583-592 (1995)

137. Fuki, I.V., K.M. Kuhn, I.R. Lomazov, V.L. Rothman, G.P. Tuszynski, R.V. Iozzo, T.L. Swenson, E.A. Fisher & K.J. Williams: The syndecan family of proteoglycans. Novel receptors mediating internalization of atherogenic lipoproteins in vitro. *J Clin Invest* 100, 1611-1622 (1997)

138. Williams, K.J. & I.V. Fuki: Cell-surface heparan sulfate proteoglycans: dynamic molecules mediating ligand catabolism. *Curr Opin Lipidol* 8, 253-262 (1997)

139. Linton, M.F., A.H. Hasty, V.R. Babaev & S. Fazio: Hepatic apoE expression is required for remnant lipoprotein clearance in the absence of the LDL receptor. *J Clin Invest* 101, 1726-1736 (1998)

140. Fazio, S., M.F. Linton, A.H. Hasty & L.L. Swift: Recyling of apolipoprotein E in mouse liver. *J Biol Chem* 274, 8247-8253 (1999)

141. Linton, M.F., V.R. Babaev, L.A. Gleaves & S. Fazio: A direct role for the macrophage low density lipoprotein receptor in atherosclerotic lesion formation. *J Biol Chem* 274, 19204-19210 (1999)

142. Hasty, A.H., M.F. Linton, L.L. Swift & S. Fazio: Determination of the lower threshold of apolipoprotein E

resulting in remnant lipoprotein clearance. *J Lipid Res* 40, 1529-1538 (1999)

143. Linton, M.F. & S. Fazio: Macrophages, lipoprotein metabolism and atherosclerosis: insights from murine bone marrow transplantation. *Curr Opin Lipidol* 10, 97-105 (1999)

144. van Dijk, K.W., J.M. van Vlijmen, B. van't Hof, S. van der Zee, S. Santamarina-Fojo, T.J.C. van Berkel, M. Havekes & M.H. Hofker: In LDL receptor-knockout mice, catabolism of remnant lipoproteins requires a high level of apoE but is inhibited by excess apoE. *J Lipid Res* 40, 336-345 (1999)

145. Fujioka, Y., L.G. Fong & A.D. Cooper: Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. *J Lipid Res* 39, 2339-2349 (1997)

146. Shimano, H., N. Yamada, K. Katsuki, T. Yamamoto, K. Gotoda, M. Harada, Y. Shimada & Y. Yazaki: Plasma lipoprotein metabolism in transgenic mice overexpress apolipoprotein E. Accelerated clearance of lipoproteins containing apolipoprotein B. *J Clin Invest* 90, 2084-2091 (1992)

147. Stevenson, S.C., J. Marshall-Neff, B. Teng, C.B. Lee, S. Roy & B. McClelland: Phenotypic correction of hypercholesterolemia in apoE-deficient mice by adenovirus-mediated in vivo gene transfer. *Arteroscler Thromb.Vasc Biol* 15, 479-484 (1995)

148. Tsukamoto, K., P. Smith, J.M. Glick & D.J. Rader: Liver directed gene transfer and prolonged expression of three major human apoE isoforms in apoE-deficient mice. *J Clin Invest* 100, 107-114 (1997)

149. Rensen, P.C.N. & T.J.C. van Berkel: Apolipoprotein E effectively inhibits lipoprotein lipase-mediated lipolysis of chylomicron-like triglyceride-rich lipid emulsions in vitro and in vivo. *J Biol Chem* 271, 14791-14799 (1996)

150. Daggy, B.P. & A. Bensadoun: Enrichment of apolipoprotein B-48 in the LDL density class following in vivo inhibition of hepatic lipase. *Biochim Biophys Acta* 877, 252-261 (1986)

151. Demacker, P.N., A.G. Hijmans, A.F. Stalenhoef & A. van't Laar: Studies on the function of hepatic lipase in the cat after immunological blockade of the enzyme in vivo. *Atherosclerosis* 69, 1730183 (1988)

152. Sultan, F., D. Lagrange, X. Le liepvre & S. Griglio: Chylomicron-remnant uptake by freshly isolated hepatocytes. Effect of heparin and of hepatic triacylglycerol lipase. *Biochem J* 258, 587-594 (1989)

153. Sultan, F., D. Lagrange, H. Jansen & S. Griglio: Inhibition of hepatic lipase activity imparis chylomicron remnant removal in rats. *Biochim Biophys Acta* 1042, 150-152 (1990)

154. Shafi, S., S.E. Brady, A. Bensadoun & R.J. Havel: Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J Lipid Res* 35, 709-720. (1994)

155. Amar, M.L., K.A. Dugi, C.C. Haundenschild, R.D. Shamburek, B. Foger, M. Chase, A. Bensadoun, R.F. Hoyt, H.B. Brewer, Jr. & S. Santamarina-Fojo: Hepatic lipase facilitates the selective uptake of cholesteryl esters from remnant lipoproteins in apoE-deficient mice. *J Lipid Res* 39, 2436-2442 (1998)

156. Choi, S.Y., I.J. Goldberg, L.K. Curtiss & A.D. Cooper: Interaction between apoB and hepatic lipase mediates the uptake of apoB-containing lipoproteins. *J Biol Chem* 273, 20456-20462 (1998)

157. Santamarina-Fojo, S., C. Haudenschild & M. Amar: The role of hepatic lipase in lipoprotein metabolism and athersclerosis. *Curr Opin Lipidol* 9, 211-219 (1998)

158. Connelly, P.W.: The role of hepatic lipase in lipoprotein metabolism. *Clinical Chemistry Acta* 286, 243-255 (1999)

159. Cohen, J.C., G.L. Vega & S.M. Grundy: Hepatic lipase: new insights from genetic and metabolic studies. *Curr Opin Lipidol* 10, 259-267 (1999)

160. Qiu, S., N. Bergeron, L. Kotite, R.M. Krauss, A. Bensadoun, A. & R.J. Havel: Metabolism of lipoproteins containing apolipoprotein B in hepatic lipase-deficient mice. *J Lipid Res* 39, 1661-1668 (1998)

161. Borensztajn, J. & T.J. Kotlar: Hepatic uptake of phospholipid-depleted chylomicros *in vivo*. Comparison with the uptake of chylomicron remnants. *Biochem. J.* 200, 547-553 (1981)

162. Borensztajn, J., G.S. Getz & T.J. Kotlar: Uptake of chylomicron remnatns by the liver: further evidence for the modulating role of phospholipids. *J Lipid Res* 29, 1087-1096 (1988)

163. Borensztajn, J., T.J. Kotlar & S.Y. Chang: Apoprotein-independent binding of chylomicron remnants. *Biochem. J.* 279, 769-773 (1991)

164. Crawford, S.E. & J. Borensztajn: Plasma clearance and liver uptake of chylomicron remnatns generated by hepatic lipase lipolysis: evidence for a lactoferrin-sensitive and apolipoprotein E-independent pathway. *J Lipid Res* 40, 797-805 (1999)

165. Felts, J.M., H. Itakura & R.T. Crane: The mechanism of assimilation of constituents of chylomicrons, very low density lipoproteins and remnants-a new theory. *Biochem Biophys Res Comm* 66, 1467-1475 (1975)

166. Beisiegel, U., W. Weber & O. Bengtsson-Olivecrona: Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein-related protein. *Proc Natl Acad Sci USA* 88, 8342-8346 (1991)

167. Krapp, A., H. Zhang, D. Ginzinger, M.S. Liu, A. Lindberg, G. Olivecrona, M.R. Hayden & U. Beisiegel: Structural features in lipoprotein lipase necessary for the mediation of lipoprotein uptake into cells. *J Lipid Res* 36, 2362-2373 (1995)

168. Nykjaer, A., G. Bengtsson-Olivecrona, A. Lookene, S.K. Moestrup, C.M. Petersen, W. Weber, U. Beisiegel & J. Gliemann: The alpha-2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and beta-migrating very low density lipoprotein associated with the lipase. *J Biol Chem* 268, 15048-15055 (1993)

169. Chappell, D.A., G.L. Fry, M.A. Waknitz, L.E. Muhonen & M.W. Pladet: Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha-2-macroglobulin receptor *in vivo*. *J Biol Chem* 268, 14168-14175 (1993)

170. Zambon, A., I. Schmidt, U. Beisiegel & J.D. Brunzell: Dimeric lipoprotein lipase is bound to triglyceride-rich plasma lipoproteins. *J Lipid Res* 37, 2394-23404 (1996)

171. Karpe, F., S.M. Humphreys, J.S. Samra, L.K. Summers & K.N. Frayn: Clearance of lipoprotein remnant particles in adipose tissue and muscle in humans. *J Lipid Res* 38, 2335-2343 (1997)

172. Karpe, F., T. Olivecrona, G. Olivecrona, J.S. Samra, L.K. Summers, S.M. Humphreys & K.N. Frayn: Lipoprotein lipase transport in plasma: role of muscle and adipose tissue in regulation of plasma lipoprotein lipase concentrations. *J Lipid Res* 39, 2387-2393 (1998)

173. van Dijk, M.C, G.J. Ziere, W. Boers, C. Linthorst, M.K. Bijsterbosch & T.J. van Berkel: Recognition of chylomicron remnatns and beta-migrating very-low-density-lipoproteins by the remnant receptor of parenchymal liver cells is dinstinct from the liver alpha 2-macroglobulin-recognition site. *Biochem J* 279, 863-870 (1991)

174. van Dijk, M.C., J.K. Kruijt, C. Boers, C. Linthorst & T.J.C. van Berkel: Distinct properties of the recognition sites for beta-very-low-density-lipoprotein receptor (remnant receptor) and alpha-2-macroglobulin (low density lipoprotein-related protein) on rat parenchymal cells. *J Biol Chem* 267, 17732-17737 (1992)

175. Ziere, G.J., M.E. van der Kaaden, C.J. Vogelezang, W. Boers, B.E. Bihain, J. Kuiper, J.K. Kruijt & T.J.C. van Berkel: Blockade of the alpha-2-macroglobulin receptor/low-density-lipoprotein receptor-related protein on rat liver parenchymal cells by the 39-kDa receptor-associated protein leaves the interaction of beta-migrating very-low-density-lipoprotein with the lipoprotein remnant receptor unaffected. *Eur J Biochem* 242, 703-711 (1996)

176. Ginsberg, H.N., W. Karmally, S. Siddiqui, S. Holleran, A.R. Tall, S.C. Ramsey, R.J. Deckelbaum, W.W. Blaner and R. Ramakrishnan: A dose-response study of the effects of dietary cholesterol on fasting and postprandial lipid and lipoprotein metabolism in healthy young men. *Arter Thromb* 14, 576-586 (1994)

177. Clifton, P. & P.J. Nestle: Effect of dietary cholesterol on postprandial lipoproteins in three phenotypic groups. *Am J Clin Nut* 64, 361-367 (1996)

178. Ross, R.: The pathogenesis of atherosclerosis - an update. *New Engl J Med* 314, 488-500 (1986)

179. Ross, R.: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362, 801-109 (1993)

180. Campbell, J.H. & G.R. Campbell: The role of smooth muscle cells in atherosclerosis. *Curr Opin Lipidol* 5, 323-330 (1994)

181. Gerrity, R.G.: The role of the monocyte in atherogenesis. *Am J Pathol* 103, 181-190 (1981)

182. Libby, P.: Atheroma: more than mush. *Lancet* 348 (suppl 1), s4-s7 (1996)

183. Frank, J.S. & A.M. Fogelman: Ultrastructure of the intima in WHHL and cholesterol-fed rabbit aortas prepared by ultra-rapid freezing and freeze-etching. *J Lipid Res* 30, 967-978 (1989)

184. Schwenke, D.C. & T.E. Carew: Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. *Arteriosclerosis* 9, 895-907 (1989)

185. Williams, K.J. & I. Tabas: The response-to-retention hypothesis of early atherogenesis. *Arter Thromb Vasc Biol* 15, 551-561 (1995)

186. Guyton, J.R. & K.F. Klemp: Development of the lipid-rich core in human atherosclerosis. *Arterosclerosis Thrombosis and Vascular Biology* 16, 4-11 (1996)

187. Steinberg, D., S. Parthasarthy, T.E. Carew, J.C. Khoo & J.L. Witztum: Beyond cholesterol: modification of low-density lipoprotein that increases its atherogenicity. *New Engl J Med* 320, 915-923 (1989)

188. Badwey, J.A. & M.L. Karnovsky: Active oxygen species and the functions of phagocytic leukocytes. *Ann Rev Biochem* 49, 695-726 (1980)

189. Chait, A. & J.W. Heinecke: Lipoprotein modifcation: cellular mechanisms. *Curr Opin Lipidol* 5, 365-370 (1994)

190. Parthasarathy, S., N. Santanam, S. Ramachandran & O. Meilhac: Oxidants and antioxidants in atherogenesis. An appraisal. *J Lipid Res* 40, 2143-2157 (1999)

191. Nordestgaard, B.G.: The vascular endothelial barrier-selective rentention of lipoproteins. *Curr Opin Lipidol* 7, 269-273 (1996)

192. Palade, G.E., M. Simionescu & N. Simionescu: Structural asepcts of the permeability of the microvascular endothelium. *Acta Physio Scand* 463 (suppl.), 11-32 (1979)

193. Sztul, E., A. Kaplin, L. Saucan & G. Palade: Protein tranffic between distinct plasma membrane domains: isolation and characterization of vesicular carriers involved in transcytosis. *Cell* 64, 81-89 (1991)

194. Predescu, D., S. Predescu, T. McQuistan, & G.E. Palade: Transcytosis of alpha-1-acidic glycoprotein in the continuous microvascular endothelium. *Proc Natl Acad Sci USA* 95, 6175-6180 (1998)

195. Simionescu, N., M. Simionescu & G.E. Palade: Structural basis of permeability in sequential segments of the microvasculature of the diaphragm. II. Pathways followed by microperoxidase across the endothelium. *Microvascular Res* 15, 17-36 (1978)

196. Simionescu, N., R. Mora, E. Vasile, F. Lupu, D.A. Filip & M. Simionescu: Prelesional modificatons of the vessel wall in hyperlipidemic atherogenesis. Extracellular accumulation of modified and reassembled lipoproteins. *Ann NY Acad Sci* 598, 1-16 (1990)

197. Newman, H.A.I. & D.B. Zilversmit: Quantitative aspects of cholesterol fluc in rabbit atheromatous lesions. *J Biol Chem* 237, 2078-2984 (1962)

198. Newman, H.A. & D.B. Zilversmit: Uptake and release of cholesterol by rabbit atheromatous lesions. *Circ Res* 18, 293-302 (1966)

199. Mamo, J.C.L. & J.R. Wheeler: Chylomicrons or their remnants penetrate rabbit thoracic aortas as efficiently as smaller macromolecules including low density lipoproteins, high density lipoproteins and albumin. *Cor Art Dis* 5, 695-705 (1994)

200. Mamo, J.C.L., S.D. Proctor & D. Smith: Retention of chylomicron remnants by arterial tissue: importance of an efficient clearance mechanism from plasma. *Atherosclerosis* 141 (suppl), S63-S69 (1998)

201. Rapp, J.H., A. Lespine, R.L. Hamilton, N. Colyvas, A.H. Chaumeton, J. Tweedie-Hardman, L. Kotite, S.T. Kunitake, R.J. Havel, & J.P. Kane: Triglyceride-rich lipoproteins isolated by selective-affinity anti-apolipoprotein B immunsorbtion from human atherosclerotic plaque. *Arter Thromb* 14, 1767-1774 (1994)

202. Li, H., M.I. Cybulsky, M.A. Gimbrone, Jr. & P. Libby: An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arter Thromb* 13, 197-204 (1993)

203. Johnson-Tidey, R.R., J.L. Gregor, P.R. Taylor & R.N. Poston: Increase in the adhesion molecule P-selectin in endothelium overlying atherosclerotic plaques: coexpression with intercellular adhesion molecule-1. *Am J Pathol* 144, 952-961 (1994)

204. Muller, W.A., S.A. Weigl, X. Deng, X. & D.M. Philips: PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 178, 449-460 (1993)

205. Pizcueta, P. & F.W. Luscinskas: Monoclonal antibody blockade of L-selectin inhibits mononuclear leukocyte recruitment in inflammatory sites in vivo. *Am J Pathol* 145, 461-469 (1994)

206. Lefer, D.J. & D.N. Granger: Monocyte rolling in early atherogenesis: vital role in lesion development. *Circ Res* 84, 1353-1355 (1999)

207. Carlos, T.M., A. Dobrina, R. Ross & J.M. Harlan: Multiple receptors on human moncocytes are involved in adhesion to cultured endothelial cells. *J Leuk Biol* 48, 451-456 (1990)

208. Springer, T.A.: Adhesion receptors of the immune system. *Nature* 346, 425-434 (1990)

209. Poston, R.N., D.O. Haskard, J.R. Coucher, N.P. Gall, & R.R. Johnson-Tidey: Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* 140, 665-673 (1992)

210. Tedder, T.F., D.A. Steeber, A. Chen & P. Engel: The selectins: vascular adhesion molecules. *FASEB J.* 9, 866-873 (1995)

211. Masuda, I. & R. Ross: Atherogenesis during low level hypercholesterolemia in the non-human primate. 1. fatty streat conversion to fibrous plaque. *Arteriosclerosis* 10, 178-187 (1990)

212. Faggiotto, A., R. Ross, & L. Harker: Studies of hypercholesterolemia in the nonhuman primate.1. Changes that lead to fatty streak formation. *Arteriosclerosis* 4, 323-340 (1984)

213. Muller, W.A. & G.J. Randolph: Migraton of leukocytes across endothelium and beyond: molecules involved in the transmigration and fate of monocytes. *J Leuk Biol* 66, 698-704 (1999)

214. Allen, S., S. Khan, F. Al-Mohanna, P. Batten & M. Yacoub: Native low density lipoprotein-induced calcium transients trigger VCAM-1 and E-selectin expression in cultured human vascular endothelial cells. *J Clin Invest* 101, 1064-1075 (1998)

215. Cockerill, G.W., J. Saklatvala, S.H. Ridley, H. Yarwood, N.E. Miller, B. Oral, S. Nithyanathan, G. Taylor, & D.O. Haskard: High-density lipoproteins differentially modulate cytokine-induced expression of E-selectin and

cyclooxygenase-2. *Arter Thromb Vasc Biol* 19, 910-917 (1999)

216. Hennig, B., B.H. Chung, B.A. Watkins & A. Alvarado: Disruption of endothelial barrier function by lipolytic remnants of triglyceride-rich lipoproteins. *Atherosclerosis* 95, 235-247 (1992)

217. Weinstein, D.B., T.E. Carew & D. Steinberg: Uptake and degradaton of low density lipoproteins by swine arterial smooth muscle cells with inhibition of cholesterol biosynthesis. *Biochim Biophys Acta* 424, 404-421 (1976)

218. Floren, C-H., J.J. Albers & E.L. Bierman: Uptake of chylomicron remnants causes cholesterol accumulation in cultured human arterial smooth muscle cells. *Biochim Biophys Acta* 663, 336-349 (1981)

219. Chen, Q., C-H. Floren, A. Nilsson, A. and R. Infante: Regulation of chylomicron remnant uptake in human hepatoma cell line HepG2. Role of the low-density lipoprotein receptor. *Biochim Biophys Acta* 1083, 173-178 (1991)

220. Yu, K.C-W. & J.C.L. Mamo: Binding and uptake of chylomicron remnants by cultured arterial smooth muscle cells from normal and Watanabe-heritable-hyperlipidemic rabbits. *Biochim Biophys Acta* 1346, 212-220 (1997)

221. Acton, S.L., P.E. Scherer, H.F. Lodish & M. Krieger: Expression cloning of SR-B1, a CD36-related class B scavenger receptor. *J Biol Chem* 269, 21003-21009 (1994)

222. Ramprasad, M.P., V. Terpstram, N. Kondratenko, O. Queherberger & D. Steinberg: Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 93, 14833-14838 (1996)

223. de Villiers, W.J. & E.J. Smart: Macrophage scavenger receptors and foam cell formation. *J Leuk Biol* 66, 740-746 (1999)

224. Yu, K.C-W. & J.C.L. Mamo: Regulation of cholesterol synthesis and esterification in primary cultures of macrophages following uptake of chylomicron remnants. *Biochem Mol Biol Int* 41, 33-39 (1997)

225. Ellsworth, J.L., A.D. Cooper & F.B. Kraemer: Evidence that chylomicron remnants and beta-VLDL are transported by the same receptor pathway in J774 murine macrophage-derived cells. *J Lipid Res* 27, 1062-1072 (1986)

226. Georgopoulos, A., S.D. Kafonek & I. Raikhel: Diabetic postprandial triglyceride-rich lipoproteins increase cholesterol accumulation in THP-1 macrophages. *Metabolism* 43, 1063-1072 (1994)

227. Yu, K.C-W. & J.C.L. Mamo: Chylomicron-remnant-induced foam cell formation and cytotoxicity: a possible mechanism of cell death in atherosclerosis. *Clin Sci* 98, 184-192 (2000)

228. Floren, C-H. & A. Chait: Uptake of chylomicron remnants by the native LDL receptor in human monocyte-derived macrophages. *Biochim Biophys Acta* 665, 608-611 (1981)

229. Ellsworth, J.L., F.B. Kraemer & A.D. Cooper: Transport of beta-very low density lipoproteins and chylomicron remnants by macrophages is mediated by the low density lipoprotein receptor pathway. *J Biol Chem* 262, 2316-2325 (1987)

230. Ellsworth, J.L., L.G. Fong, F.B. Kraemer & A.D. Cooper: Differences in the processing of chylomicron remnants and beta-VLDL by macrophages. *J Lipid Res* 31, 1399-1411 (1990)

231. Tabas, I., D.A. Weiland & A.R. Tall: Unmodified low density lipoprotein causes cholesteryl ester accumulation in J774 macrophages. *Proc Natl Acad Sci USA* 89, 9252-9256 (1985)

232. Tabas, I., J.N. Myers, T.L. Innerarity, X.X. Xu, K. Arnold, J. Boyles & F.R. Maxfield: The influence of particle size and multiple apoprotein E-receptor interactons on the endocytic targeting of beta-VLDL in mouse peritoneal macrophages. *J Cell Biol* 115, 1547-1560 (1991)

233. Mahley, R.W., T.L. Innerarity, M.S. Brown, Y.K. Ho & J.L. Goldstein: Cholesteryl ester synthesis in macrophages: stimulation by beta-very-low-density-lipoproteins from cholesterol-fed animals of several species. *J Lipid Res* 21, 970-980 (1980)

234. Mamo, J.C.L., C.L. Elsegood, H.C. Gennat & K.C-W. Yu: Degradation of chylomicron remnants by macrophages occurs via phagocytosis. *Biochemistry* 35, 10210-10214 (1996)

235. Hiesberger, T., D. Huttler, A. Rohlmann, W. Schneider, K. Sandhoff & J. Herz: Cellular uptake of saposin (SAP) precursor and lysosomal delivery by the low density receptor-related protein (LRP) *EMBO J.* 17, 4617-4625 (1998)

236. Misra, U.K., G. Gawdi, M. Gonzalez-Gronow & S.V. Pizzo: Coordinate regulation of the alpha2-macroglobulin signaling receptor and the low density lipoprotein receptor-related protein/alpha-2-macroglobulin receptor by insulin. *J Biol Chem* 274, 25785-25791 (1999)

237. Ramprasad, M.P., R. Li, S.H. Gianturco & W.A. Bradley: Purification of the human THP-1 monocyte-macrophage triglyceride-rich lipoprotein receptor. *Biochem Biophys Res Comm* 210, 491-497 (1995)

238. Gianturco, S.H., M.P. Ramprasad, R. Song, R. Li, M.L. Brown & W.A. Bradley: Apolipoprotein B-48 or its apolipoprotein B-100 equivalent mediates the binding of triglyceride-rich lipoproteins to their unique human monocyte-macrophage receptor. *Arter Thromb Vasc Biol* 18, 968-976 (1998)

239. Bradley, W.A., M.L. Brown, M.O. Ramprasad, R. Li, R. Song & S.H. Gianturco: Antipeptide antibodies reveal

interrelationships of MBP200 and MBP235: unique apoB-specific receptors for triglyceride-rich lipoproteins on human monocyte-macrophages. *J Lipid Res* 40, 744-752 (1999)

240. Albrink, M.J. & E.B. Man: Serum triglycerides in coronary artery disease. *Arch Int Med* 103, 4-8 (1959)

241. Patsch, J.R.: Triglyceride-rich lipoproteins and atherosclerosis. *Atherosclerosis* 110 (suppl), S23-S26 (1994)

242. Simons, L.A., T. Dwyer & J. Simon: Chylomicrons and chylomicron remnants in coronary artery disease: a case-control study. *Atherosclerosis* 65, 181-189 (1987)

243. Simpson, H.S., C.M. Williamson, T. Olivecrona, S. Pringle, J. MacLean, A.R. Lorimer, F. Bonnefous, Y. Bogaievsky, C.J. Packard & J. Shepherd: Postprandial lipemia, fenofibrate and coronary artery disease. *Atherosclerosis* 193-202, 193-202 (1990)

244. Groot, P.H.E., W.A. van Stiphout, X.H. Krauss, H. Jansen, A. van Tol, E. van Ramshorst, S. Chin-On, A. Hofman, S.R. Cresswell & L. Havekes: Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arter Thromb* 11, 653-662 (1991)

245. Karpe, F., G. Steiner, T. Olivecrona, L.A. Carlson & A. Hamsten: Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J Clin Invest* 91, 748-758 (1993)

246. Karpe, F., G. Steiner, K. Uffelman, T. Olivecrona & A. Hamsten: Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 106, 83-97 (1994)

247. Ginsberg, H.N., J. Jones, W.S. Blamer, A. Thomas, W. Karmally, L. Fields, D. Blood & M.D. Begg: Association of postprandial triglyceride and retinyl palmitate responses with newly diagnosed exercised-induced myocardial ischemia in middle-aged men and women. *Arter Thromb Vasc Biol* 15, 1829-1838 (1995)

248. Weintraub, M.S., I. Grosskopf, T. Rassin, H. Miller, G. Charach, H.H. Rotmensch, M. Liron, A. Rubinsteinm & A. Iaina: Clearance of chylomicron remnants in normolipidaemic patients with coronary artery disease: case control study over three years. *Brit Med J* 312, 936-939 (1996)

249. Meyer, E., H.T. Westerveld, F.C. de Ruyter-Meijstek, M.M. van Greevenbroek, R. Rienks, H.J. van Rijn, D.W. Erkelens,& T.W. de Bruin: Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case-control study. *Atherosclerosis* 124, 221-235 (1996)

250. Weintraub, M.S., I. Grosskopf, I., G. Charach, N. Eckstein & A. Rubinstein: Hormone replacement therapy enhances postprandial lipid metabolism in postmenopausal women. *Metabolism* 48, 1193-1196 (1999)

251. van Beek, A.P., F.C. de Tuijter-Heijstek, D.W. Erkelens & T.W. de Bruin: Menopause is associated with reduced protection from postprandial lipemia. *Arter Thromb Vasc Biol* 19, 2737-2741 (1999)

252. Cabezas, M.C., T.W. de Bruin, H.E. Westerveld, E. Meijer & D.W. Erkelens: Delayed chylomicron remnant clearance in subjects with heterozygous familial hypercholesterolemia. *J Int Med* 244, 299-307 (1998)

253. Eriksson, M., B. Angelin, P. Henriksson, S. Ericsson, S. Vitols & L. Berglund: Metabolism of lipoprotein remnants in humans. Studies during intestinal infusion of fat and cholesterol in subjects with varying expression of low density lipoprotein receptor. *Arter Thromb* 11, 827-837 (1991)

254. Castro-Cabezas, M., T.W.A. de Bruin, H. Jensen, L.A.W. Kock, W. Kortlandt & P. Gambert: Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arter Thromb* 13, 804-814 (1993)

255. Syvanne, M., H. Vuorinen-Markkola, H. Hilden & M.R. Taskinen: Gemfibrozil reduces postprandial lipemia in non-insulin-dependent diabetes mellitus. *Arter Thromb* 13, 286-295 (1993)

256. Geogopoulos, A. & C.D. Saudek: Intraperitoneal insulin delivery decreases the levels of chylomicron remnants in patients with IDDM. *Diabetes Care* 17, 1295-1299 (1994)

257. Curtin, A., P. Deegan, D. Owens, P. Collins, A. Johnson & G.H. Tomkin: Elevated triglyceride-rich lipoproteins in diabetes. A study of apolipoprotein B-48. *Acta Diabet* 33, 205-210 (1996)

258. Reznik, Y., P. Pousse, M. Herrou, R. Morello, J. Mahoudeau, M.A. Drosdowsky & S.Fradin: Postprandial lipoprotein metabolism in normotriglyceridemic non-insulin-dependent diabetic patients: influence of apolipoprotein E polymorphism. *Metabolism* 45, 63-71 (1996)

259. Georgopoulos, A., J.P. Bantle, M. Noutsou, W.R. Swaim & S.J. Parker: Differences in the metabolism of postprandial lipoproteins after a high-monosaturated versus a high-carbohydrate diet in patients with type 1 diabetes mellitus. *Arter Thromb Vasc Biol* 18, 773-782 (1998)

260. Mero, N., M. Syvanne & M.R. Taskinen: Postprandial lipid metabolism in diabetes. *Atherosclerosis* 141 (Suppl), S53-S55 (1998)

261. Noutsou, M. & A. Georgopoulos: Effects of simvastatin on fasting and postprandial triglyceride-rich lipoproteins in patients with type 1 diabetes mellitus. *J Diabetes Comp* 13, 98-104 (1999)

262. Reaven, G.M.: Role of insulin resistance in human disease (syndrome X): an expanded definition. *Ann Rev Med* 44, 121-131 (1993)

263. Reaven, G.M.: Syndrome X: is one enough? *Am Heart J* 127, 1439-1442 (1994)

264. Reaven, G.M.: Pathophysiology of insulin resistance in human disease. *Physiol Rev* 75, 473-486 (1995)

265. Chen, Y.D., A.M. Coulston, M.Y. Zhou, C.B. Hollenbeck & G.M. Reaven: Why do low-fat high-carbohydrate diets accentuate postprandial lipemia in patients with NIDDM? *Diabetes Care* 18, 10-16 (1995)

266. Jeppesen, J., C.B. Hollenbeck, M.Y. Zhou, C. Jones, Y.D. Chen & G.M. Reaven: Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity and postprandial lipemia. *Arter Thromb Vasc Biol* 15, 320-324 (1995)

267. Abbasi, F., T. McLaughlin, C. Lamendola, H. Yeni-Komshian, A. Tanaka, T. Wang, K. Nakajima & G.M. Reaven: Fasting remnant lipoprotein cholesterol and triglyceride concentratons are elevated in nondiabetic, insulin-resistant, female volunteers. *J Clin Endocrin Metab* 84, 3903-3906 (1999)

268. Ross, R.: Atherosclerosis is an inflammatory disease. *Am Heart J* 138, S419-S420 (1999)

269. Galle, J., Heermeier, K & C. Wanner: Atherogenic lipoproteins, oxidative stress, and cell death. *Kidney Int* 56 (suppl), (1999)

270. Schroepfer, G.J. Jr: Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol Rev* 80, 361-554 (2000)

271. Zilversmit, D.B.: Atherogenic nature of triglycerides, postprandial lipidemia and triglyceride-rich remnant lipoproteins. *Clin Chem* 41, 153-158 (1995)

272. Schwartz, S.M. & C.E. Murry: Proliferation and the monoclonal origins of atherosclerotic lesions. *Ann Rev Med* 49, 437-460 (1998)

273. Gerhard, G.T. & P.B. Duell: Homocysteine and atherosclerosis. *Curr Opin Lipidol* 10, 417-428 (1999)

274. Shor, A. & J.L. Philips: Chlamydia pneumoniae and atherosclerosis. *J Am Med Assoc* 282, 2071-2073 (1999)

275. Campbell, L.A. & C. Kuo: Mouse models of Chlamydia pneumoniae and atherosclerosis. *Am Heart J* 138, (1999)

276. Hobbs, H.H. & A.L. White: Lipoprotein(a): intrigues and insights. *Curr Opin Lipidol* 10, 225-236 (1999)

277. Kim, J.A., J.A. Berliner, R.D. Natarajan & J.L. Nadler: Evidence that glucose increases monocyte binding to human endothelial cells. *Diabetes* 43, 1103-1107 (1994)

278. Hoogerbrugge, N., A. Verkerk, M.L. Jacobs, P.T. Postema & J.F. Jongkind: Hypertriglyceridemia enhances monocyte binding to endothelial cells in NIDDM. *Diabetes Care* 19, 1122-1125 (1996)

279. Tsao, P.S., J. Nieubauer, R. Buitrago, P.S. Liu, B.T. Wang, J.P. Cooke, Y.D. Chen & Reaven, G.M.: Interaction of diabetes and hypertension on determinants of endothelial adhesiveness. *Arter Thromb Vasc Biol* 18, 947-953 (1998)

280. Gerrity, R.G., H.K. Naito, M. Richardson & C.J. Schwartz: Dietary induced atherogenesis in swine: morphology of the intima in prelesion stage. *Am J Path* 95, 775-792 (1979)

281. Yu, K.C-W. & J.C.L. Mamo: Killing of arterial smooth muscle cells by chylomicron remnants. *Biochem Biophys Res Commun* 220, 68-71 (1996)

282. Chung, B.H., G.A. Tallis, S.B.H. Cho, J.P. Segrest & Y.Henkin: Lipolysis-induced partitioning of free fatty acids to lipoproteins: effects of the biological properties of free fatty acids. *J Lipid Res* 36, 1956-1970 (1995)

283. Mitropoulos, K.A., G.J. Miller, B.E.A. Reeves, H.C. Wilkes & J.K. Cruick-Shank: Factor VII coagulant activity is strongly associated with the plasma concentration of large lipoprotein particles in middle-aged men. *Atherosclerosis* 76, 203-208 (1989)

284. Miller, G.J., J.C. Martin, K.A. Mitropoulos & B.E.A. Reeves: Plasma factor VII is activated by postprandial triglyceridemia, irrespective of dietary fat consumption. *Atherosclerosis* 86, 163-171 (1991)

285. Mitropoulos, K.A., G.J. Miller, J.C. Martin, B.E.A. Reeves & J. Cooper: Dietary fat induces changes in factor VII coagulant activity through effects on plasma free stearic acid concentration. *Arter Thromb* 14, 214-222 (1994)

286. Frolkis, J.P.: Screening for cardiovascular disease: concepts, conflicts and consensus. *Med Clin North Am* 83, 1339-1373 (1999)

287. Grundy, S.M.: Cholesterol management in the era of managed care. *Am J Cardiol* 85, 3A-9A (2000)

Keywords: Liver, Cells, Chylomicrons, Chylomicron Remnants, Triglyceride-Rich Lipoproteins, Apolipoprotein E, Coronary Heart Disease, LDL Receptor, LDL Receptor-Related-Protein, Heparan Sulfate Proteoglycans, Hepatic Lipase, Endothelial Smooth Muscle Cells, Macrophages, Review

Send correspondence to: Dr. Allen D. Cooper, M.D., Research Institute, Ames Building, Palo Alto Medical Foundation, 795 El Camino Real, Palo Alto,CA 94301, USA. Tel: 650-853-8120, Fax: 650-329-9114, E-mail: adc@leland.stanford.edu