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Lipoprotein metabolism in non-insulin-dependent diabetes mellitus

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The risk and incidence of vascular disease in patients with non-insulin-dependent diabetes mellitus (NIDDM) are higher than those of the nondiabetic population. The modest changes in the concentration of plasma lipids that have been reported do not fully explain this increased risk of vascular disease in diabetics. However, there have been numerous reports of changes in the composition and structure of plasma lipoproteins in patients with NIDDM that render these lipoproteins more atherogenic. Changes in the subpopulation distribution of plasma low-density lipoprotein and high-density lipoprotein, which have been shown to be associated with vascular disease, have been also observed in patients with NIDDM. Although the precise mechanisms that underlie the origination of alterations in the subpopulation distribution of plasma lipoproteins have not been fully elucidated, evidence strongly suggests that changes in the activities of key enzymes in lipoprotein metabolism may contribute to the altered lipoproteins. This review describes the effects of diabetes, along with the confounding effects of obesity and insulin resistance, on the metabolism of plasma lipoproteins. In addition, the changes in the function of enzymes that are involved in the metabolism of cholesterol and triglycerides of circulating lipoproteins that occur in NIDDM are presented. Although new insight on the effects of diabetes and its complications on the metabolism of lipoproteins has been gained, there are still gaps that need to be filled to complete our understanding of the strong relationship between diabetes and vascular disease. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:586–598, 1996.)

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Introduction

Numerous studies have demonstrated that the risk and incidence of coronary heart disease (CHD) and vascular disease in patients with diabetes mellitus are higher than those of non-diabetics.[1–11] In fact, vascular disease accounts for more than 60% of the morbidity and mortality of diabetes. Vascular disease includes both micro- and macrovascular diseases, and is common among diabetic patients whether they have juvenile or adult onset diabetes mellitus. Juvenile diabetes, or type I diabetes mellitus, occurs at a relatively young age where the beta cells of the pancreas do not produce sufficient amounts of insulin to maintain euglycemia in the plasma. This type of diabetes is referred to as insulin-dependent diabetes mellitus, or IDDM, because insulin has to be administered to maintain euglycemia. Microvascular disease is quite common among patients with this type of diabetes.

Adult onset, or type II, diabetes mellitus is also referred to as non–insulin-dependent diabetes mellitus (NIDDM). The hallmark of NIDDM is the resistance of peripheral tissues to insulin action. As a result, plasma glucose levels

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are elevated in patients with NIDDM. Accompanying the elevation in plasma glucose levels is the elevation in plasma insulin concentration, which is a compensatory mechanism to maintain euglycemia. Thus, when examining the effects of diabetes on metabolic processes, it is imperative that the additional effects of obesity and insulin resistance are also considered.

It is established now that CHD is associated with changes in plasma lipid concentrations, high blood pressure, and other risk factors. Studies have shown a positive correlation between plasma total cholesterol or low-density lipoprotein (LDL)-cholesterol levels, but an inverse correlation between levels of high-density lipoprotein (HDL)-cholesterol and the incidence of CHD. In patients with NIDDM, elevations of plasma total cholesterol and LDL-cholesterol and decreases in HDL cholesterol levels have been reported. However, these changes are modest and, hence, do not fully explain the high incidence of CHD and vascular disease in patients with NIDDM. It follows that there may be other factors that contribute to the enhanced risk and incidence of vascular disease in these patients.

Evidence shows that alterations in the structure and/or the subpopulation distribution of plasma lipoproteins may have a role in CHD.[12–22] Chemical and physical characterization of the lipoproteins have revealed that structural differences among the subfractions in a given class of lipoprotein particles may differ among individuals according to risk of CHD.[23,24] Several investigations[25–34] have examined the chemical, physical, and biological characteristics of plasma lipoproteins in patients with NIDDM. The rationale behind these studies was that changes in the properties of the lipoproteins would lead to aberrations in their metabolism and contribute to the higher incidence of CHD. Efforts were then directed at examining the underlying causes of the alterations in the characteristics of the lipoproteins. These investigations provided new insights into plausible biological causes of the enhanced prevalence of CHD in patients with NIDDM.

Reviews describing the effects of diabetes on the metabolism of the individual lipoprotein classes and other aspects of vascular disease have appeared[8,35–39] and the reader is referred to these reviews for more details. In this review, we first describe the normal metabolism of each of the classes of plasma lipoproteins and how the normal metabolism of the lipoproteins is affected by NIDDM and its attendant metabolic derangements (insulin resistance and obesity). In addition, the effects of diabetes on the function of the key enzymes in lipoprotein metabolism will be reviewed.

I. Metabolism of very-low-density lipoproteins

Very-low-density lipoproteins (VLDL) are triglyceride-rich plasma lipoproteins whose lipid and protein components are synthesized in the liver and whose function is to deliver lipids (mainly triglycerides) from the liver to peripheral tissues. They are composed of a spectrum of particles ranging in size from 30 to 80 nM and density from 0.930 to 1.006 g/mL. The chemical composition, as percent of weight, of VLDL is as follows: 9% protein, 20% phospholipids, 10% cholesterol esters, 6% unesterified cholesterol, and 55% triglycerides. The major protein components of VLDL are apolipoproteins B100, C, and E (apo B-100, apo C, and apo E, respectively). The fatty acids that are used in triglyceride and phospholipid synthesis in the liver are derived from non-esterified fatty acids in the plasma, hydrolysis of the

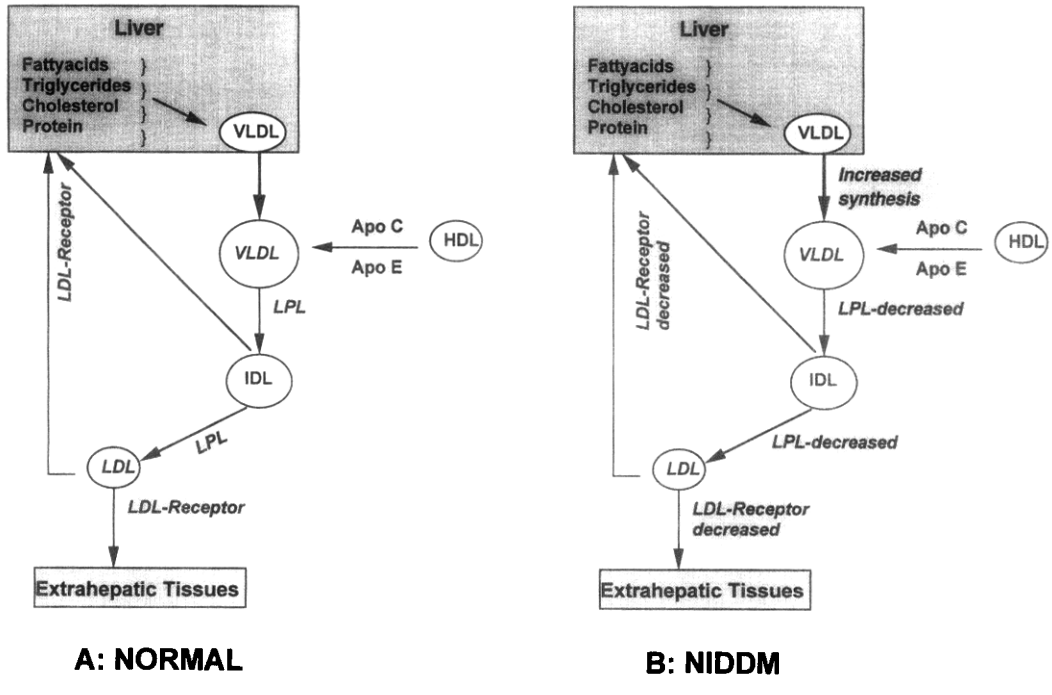

Figure 1 VLDL metabolism in normal (A) and diabetic (B) humans. Apo C = apolipoprotein C; Apo E = apolipoprotein E; LPL = lipoprotein lipase.

lipids that are returned to the liver by other lipoproteins, and from de novo synthesis from acetyl-CoA. Cholesterol is derived from either de novo synthesis in the liver, or from plasma lipoproteins that return to the liver for further metabolism (*Figure 1A*).

Triglyceride synthesis in the liver is influenced by the nutritional state of the organism. Plasma insulin and glucagon levels, as well as the glycogen content of the liver, affect the synthesis of triglycerides.[40,41] The newly synthesized triglycerides are packaged into VLDL along with cholesterol, phospholipids, and proteins (apo B-100 and apo E) in the endoplasmic reticulum, move to the Golgi apparatus for glycosylation of the proteins, and then are transported to the plasma membrane for export.[42] ApoB-100 is a key apoprotein in VLDL metabolism and alterations in its synthesis can affect plasma lipoprotein concentrations.[43] ApoB-100, a large glycosylated protein (m.w.512 kD) synthesized in the hepatocytes and secreted on VLDL, is not only necessary for the secretion of VLDL, but is also a determinant for the recapture of the catabolic products of VLDL (mostly LDL) via the LDL receptor-mediated pathway. ApoE and apoC are other apolipoproteins of VLDL that are important in its catabolism. Subsequent to hepatic secretion, the nascent VLDL receives apoC and additional apoE from HDL (*Figure 1A*).

VLDL delivers triglycerides to the tissues by interacting with lipoprotein lipase (LPL), an enzyme that is located on the endothelial cells of tissue capillaries. During the stepwise degradation of VLDL by LPL, apoC and apoE are released from the VLDL leaving the end product, LDL, with apoB-100 as the only protein on the LDL particle. The liver and extrahepatic tissues that contain the apoB and apoE receptors permit lipoprotein particles containing these apoproteins to undergo receptor-mediated endocytosis. IDL is an immediate catabolic product of VLDL, containing both apoB-100 and apoE, is more rapidly taken up by liver and other cells. Because IDLs are rapidly removed from plasma, LDL represents the major degradation end product of VLDL catabolism (*Figure 1A*).

The major function of VLDL is to deliver triglyceride-fatty acids to peripheral tissues. The hydrolysis of the fatty acids from the triglycerides of VLDL is catalyzed by the enzyme LPL. LPL from different tissues (i.e., adipose tissue and muscle), has the same characteristics in relation to substrate specificity, cofactor requirements, and releasability by heparin. However, evidence suggests that adipose tissue LPL and muscle LPL are differentially regulated, which is a reflection of their critical roles in energy metabolism in the respective tissues. Because adipose tissue LPL functions mainly in the extraction of fatty acids from circulating triglyceride-rich lipoproteins for storage, it would be expected that physiological conditions that favor storage of fat would favor a more active LPL. This is, in fact, true. Adipose tissue LPL activity increases post-prandially,[44] with carbohydrate feeding,[45] after both oral and intravenous glucose administration,[46,47] and with insulin infusion during a euglycemic clamp,[48,49] but decreases during food restriction.[46,50,51] These findings strongly suggest that insulin may have a role in modulating LPL activity. This is further supported by in vitro and in vivo evidence from experiments with rats which demonstrated that, indeed, insulin regulates LPL activity.[52–54] Further in vitro studies indicated that insulin stimulates the synthesis of enzyme protein in adipose tissue,[54,55] which was preceded by a concomitant increase in LPL-specific mRNA.[56,57]

Muscle LPL appears to be regulated in a different manner than adipose tissue LPL. In man, muscle LPL activity is lower in the fed state than in the fasted state, but LPL activity decreases during caloric restriction.[47] The effects of insulin on the regulation of muscle LPL are not clearer. Under conditions where in vivo insulin levels are expected to be high, such as during an intravenous glucose infusion, muscle LPL activity was increased.[47] In contrast, when insulin levels were decreased during exercise, LPL activity in muscle was also increased.[58] Thus, it appears that insulin alone does not have a regulatory role on LPL in human muscle. Clearly, the role of insulin in modulating muscle LPL function needs to be clarified.

One of the most consistent findings in patients with NIDDM is the elevation in plasma triglyceride content,[3,5,10,59–76] which has been attributed to increased synthesis[9,77–83] and/or decreased clearance[77,78,80–86] of VLDL (*Figure 1B*). Epidemiological studies have shown that plasma VLDL levels correlated with plasma glucose concentration, and better control of the hyperglycemia of NIDDM resulted in reduction of plasma VLDL concentrations.[77–79,82,86] Because hyperinsulinemia is another prominent feature of NIDDM, the role of insulin in causing an increase in VLDL synthesis and the subsequent elevation in plasma triglyceride levels was examined. The results showed that insulin has both a stimulatory and an inhibitory effect on VLDL synthesis and secretion. Short-term exposure of cultured hepatocytes to insulin inhibited VLDL synthesis[87–92] and increased degradation of apo-B100.[93] On the other hand, long-term exposure (>24 hr) of hepatocytes to insulin was associated with increased synthesis and secretion of VLDL into the culture medium.[87,94–98] To date, the mechanisms underlying the differences in the cellular responses of tissues to insulin, i.e., long- versus short-term exposure, have not been fully elucidated. It could be speculated, however, that long-term exposure to insulin leads to induction of lipogenic enzymes in the liver which, in turn, leads to increased TG synthesis. Evidence from our laboratory showed no difference in the activities of several hepatic lipogenic enzymes between NIDDM patients and controls.[99] Thus, it appears that the anabolic effects of insulin are not the likely cause of the elevation in VLDL-triglyceride synthesis during prolonged exposure to insulin. A more likely mechanism may be that the overexposure of cultured hepatocytes to insulin leads to insulin resistance (down-regulation of the insulin receptor), which ameliorates the inhibitory effects of insulin on TG synthesis. This may be one mechanism that is operative in NIDDM, where liver is resistant to insulin action.[100] With the resistance of tissues to insulin action, the effects of counter-regulatory hormones, such as glucagon, will lead to the mobilization of adipose tissue fatty acids and other substrates that can be used for triglyceride synthesis in the liver.

NIDDM also appears to be associated with a defect in the clearance of VLDL triglyceride resulting in hyperglyceri-

demia. Decreases in the fractional catabolic rate (FCR) of VLDL triglycerides and VLDL apo B-100 have been reported in hyperglycemic patients with NIDDM. Interestingly, better control of the hyperglycemia of NIDDM resulted in improvement in FCR. However, in some studies of hypertriglyceridemic patients with NIDDM, defects in the clearing of plasma triglycerides were not observed.[9,77,79,82] The reasons behind the lack of difference in the FCR of VLDL in hypertriglyceridemic patients are not readily apparent. However, it should be noted that the determination of FCR has been a subject of discussion among investigators, particularly with regard to subject selection and the interpretation of the results (for more details, see Ref. 35).

Experimental evidence shows that LPL function is changed in the obese or the diabetic states. Studies have shown that obesity, whether genetic or diet-induced, is associated with increases in LPL activity in humans and animal models.[101] In humans, LPL activity in adipose tissue was reported to be positively correlated to body mass index (BMI).[102] It should be noted here that in humans, obesity is usually associated with increased levels of plasma insulin. Thus, it is not yet clear whether the increase in LPL activity in adipose tissue of obese humans is due to the concomitant hyperinsulinemia or to inherited factor(s) that influence LPL function. Nonetheless, experimental evidence indicates that increased LPL activity in obese humans serves mostly to preserve the obese state rather than initiate it.[102]

Early studies of the effects of NIDDM on LPL function (*Figure 1B*) showed that NIDDM patients with hyperlipidemia have low plasma LPL activity.[103–106] In a study of normolipidemic NIDDM patients who were matched on age, sex, and body weight with a group of normolipidemic nondiabetic controls, Taskinen and coworkers[74] measured LPL activity in postheparin plasma, muscle, and adipose tissue. The results showed that LPL activity in adipose tissue of the diabetic patients was lower than that of the controls, but neither muscle nor postheparin LPL activity was different from that of the controls. These observations were confirmed by studies of Pima Indians, who are generally obese and diabetic, but not hyperlipidemic.[107] Thus, adipose tissue LPL activity is decreased in diabetic patients in the absence or presence of hyperlipidemia (*Figure 1B*). This is likely, due to the resistance of adipose tissue to insulin action in NIDDM, because treatment of diabetic patients with insulin improves the activity of LPL.[102,107] Insulin has been shown to influence the concentration of LPL protein and this control appears be at the transcriptional level.[56,57,108] It seems likely that the decreased activity of LPL in patients with NIDDM results from the resistance of tissues to insulin action, and that this decrease in LPL activity results in a decrease in the clearance of VLDL triglycerides and the concomitant rise in plasma triglyceride levels (*Figure 1B*).

Thus, the major changes in VLDL metabolism in NIDDM are the following: (a) increased synthesis of VLDL in the liver; (b) decreased clearance of VLDL-triglycerides from the circulation; and (c) decreased adipose tissue LPL activity. These changes (shown in *Figure 1B*) lead to an increase in plasma triglyceride concentration, which has been documented in NIDDM.

II. Metabolism of low-density lipoproteins

LDL are plasma lipoproteins whose role is to deliver cholesterol to the tissues. The cholesterol in the core of the LDL particle is esterified to fatty acids, and thus is delivered to the cells in the form of cholesterol esters. Cells internalize the LDL particle through a well-defined pathway, known as the LDL receptor-mediated pathway.[109]

LDL in the plasma arises mainly from VLDL after the latter lose the bulk of their core triglycerides and some of their surface proteins (*Figure 1A*). Furthermore, when circulating in the plasma, LDL particles are subjected to various processes that alter their shape and composition. These metabolic processes, collectively, will give rise to a spectrum of subpopulations of LDL particles with varying size, density, and chemical composition. LDL in the plasma have an average molecular weight of 3.0 million daltons, a density range between 1.019 and 1.063 g/mL, and a molecular diameter range between 18 and 25 nM. LDL particles contain a core of approximately 1500 cholesterol ester molecules with the most common fatty acid being linoleate. This hydrophobic core is surrounded by a monolayer of phospholipids and unesterified cholesterol. The monolayer contains a single protein, apo B-100, which is recognized by the LDL receptor on the surface of cells. LDL with these characteristics are normally metabolized by cells, where the cholesterol that is delivered to the cells will regulate the metabolism of cholesterol inside these cells. Alterations in the physical or chemical characteristics of LDL may lead to abnormalities in their metabolism, which, in turn, may influence cholesterol metabolism in cells.

There is evidence that shows that modifications and changes in the chemical composition of LDL occur in the hyperglycemic state. A prominent modification of LDL in hyperglycemia is non-enzymatic glycosylation, which is related to the concentration of glucose in the environment of the LDL particle.[110] Of importance is the demonstration that glycosylated LDL are not taken up by cultured cells as efficiently as nonglycosylated LDL because of impairments in the binding of the LDL particles to LDL receptors.[111,112] Glycosylated LDL have been reported in diabetic patients.[113–115] This suggests that cells of patients with NIDDM will not take LDL up as efficiently as cells of subjects without NIDDM, and glycosylated LDL will remain in the circulation longer and may be subjected to further modification.

Changes in the chemical composition and physical properties of LDL in patients with NIDDM have been reported by several investigators. We and others[116,117] have reported that LDL of diabetic patients were cholesterol ester-poor and triglyceride-enriched. In addition, we reported that in morbidly obese diabetic patients, there was a shift in the subpopulation distribution of plasma LDL toward a smaller and denser pattern of particle distribution compared with controls. Stewart et al.[117] reported that LDL of diabetic patients with mild hyperlipidemia had characteristics that were similar to the LDL that we observed in our patients. Because the patients in both studies were hyperinsulinemic and insulin resistant, these studies suggest that insulin re-

sistance may have a role in the development of structurally abnormal LDL.

Does obesity have a distinct role that leads to changes in the composition of LDL? We obtained evidence that showed that it may not be obesity per se that is associated with alterations in LDL composition, but rather body fat distribution may be more important in these alterations.[118] Again, body fat distribution has been shown to correlate with insulin resistance.

A great deal of interest was generated from reports that identified body fat distribution, rather than total body fat content, as being associated with several of the metabolic anomalies usually assigned to obesity.[119] Of significance was the evidence of increased insulin resistance and a predisposition to diabetes in subjects with male-type adiposity.[120–127] This type of obesity is characterized by accumulation of fat in the abdominal region. The subdivision of body fat distribution into male- and female-type obesity (apple- versus pear-shaped) allowed investigators to first dissect the effects of obesity from those of fat distribution on lipoprotein metabolism. We examined the physical and chemical properties of LDL in two groups of lean men who were matched on age, body fat content, lean body mass, and BMI, but differed in body fat distribution.[118] One group had a waist-to-hip ratio (WHR) > 1 (male-type adiposity) and the other group a WHR < 1 (female-type adiposity). We observed that LDL of the subjects with WHR > 1 were composed mainly of LDL particles that were smaller and more dense than those of the group with WHR < 1. Further, LDL of the subjects with male-type adiposity was cholesterol ester-poor and triglyceride-enriched. This study illustrated two important points: (a) alterations in the physical and chemical properties of LDL are more likely due to body-fat distribution than the degree of obesity; and (b) the changes in LDL in subjects with male-type obesity resemble those that we and others have observed in obese patients with NIDDM.

That obesity per se (percent body fat) contributes little to the alterations in LDL composition in diabetics came from other studies. We reported that LDL of morbidly obese patients without diabetes did not differ much from LDL of lean controls.[116] In addition, we observed that after the morbidly obese patients with NIDDM underwent gastric bypass surgery, their LDL reverted to a more normal pattern of composition, density, and size. Although these patients lost weight after the gastric bypass, they remained obese with an average BMI > 34. It is important to note two additional observations from these studies. First, LDL size correlated negatively, but independently, with both plasma insulin and triglyceride concentrations. Second, after the morbidly obese patients with NIDDM underwent gastric bypass surgery, their insulin sensitivity was normalized.[128] Taken together, these observations strongly suggest that insulin resistance is a major contributor to the alterations in the composition of LDL, and that total body fat content may not be as important for the alterations in LDL as body fat distribution.

The evidence of the alterations in the composition of LDL presented so far demonstrates that insulin resistance may be a major factor that contributes to the reported aberrations. This leads to two key questions: (1) What is the physiological significance of these alterations, i.e., what is the relationship between structural changes in LDL and heart disease? (2) How does the resistance of tissues to insulin action affect LDL composition or structure?

For effective binding of LDL to its receptor, the proper conformation of apo B-100 should be maintained. Alterations in the conformation of apo B-100 disturb the binding of LDL to the receptor, which leads to the prolongation of the residence of LDL in circulation. Experimental evidence suggests that the conformation of apoB-100 in small and dense LDL particles is altered. These LDL have a lower catabolic rate,[129] show decreased immunoreactivity with antibodies that recognize the apoB-receptor binding site, and have a lower affinity for the LDL receptor in cultured fibroblasts.[130] How does insulin resistance affect the biochemical processes that are associated with the metabolism of LDL? The most direct answer is that if the tissues where the enzymes and processes that are modulated by insulin and that are involved in LDL metabolism are resistant to insulin action, then these enzymes and processes will be affected by the decreased responsiveness to insulin. Thus, the major changes in plasma LDL in NIDDM include: (a) glycosylation, which leads to impairments in LDL metabolism; and (b) the prevelance of smaller and denser particles, which also are not metabolized efficiently. Both of these changes lead to the prolongation of residence time of LDL in the circulation, which, in turn, may lead to further modifications. Presumably, these changes in LDL may increase the likelihood of development of atherosclerosis in NIDDM.

III. Metabolism of high-density lipoproteins

As true of the other lipoproteins, HDL in the plasma are comprised of several populations of particles that differ in size, density, and chemical composition.[131] In the plasma, HDL are small, spherical lipoproteins that consist of approximately 50% protein and 50% lipid. Depending on their cholesterol ester content, HDL are classified mainly as the more cholesterol ester-enriched HDL_2 with a density range of 1.063–1.125 g/mL and the smaller, cholesterol ester-poor, HDL_3 with a density range of 1.125–1.210 g/mL. Although the major proportion of HDL is present as HDL_3, variations in HDL levels among individuals reflect changes in HDL_2. Thus, HDL_2 is the fraction of HDL that is usually affected by external factors.[132,133]

The sources of HDL in humans are the liver and the small intestine (*Figure 2*). Spherical HDL have been observed in liver cells. In addition, discoidal HDL, which are present in the plasma, arise extra-cellularly, as products of the delipidation of triglyceride rich lipoproteins.[133] Through the action of LPL, and after hydrolysis of the triglycerides from the triglyceride-rich lipoproteins, the phospholipid membrane doubles on itself and snips off as a discoidal double layered membrane containing small amounts of triglycerides and cholesterol esters.[132,134] This nascent HDL particle is composed mostly of phospholipids and proteins. Thus, optimal activity of LPL is necessary for the formation of HDL, and a decrease in LPL may lead to a decrease in HDL levels.

As shown in *Figure 2*, the nascent HDL particles get

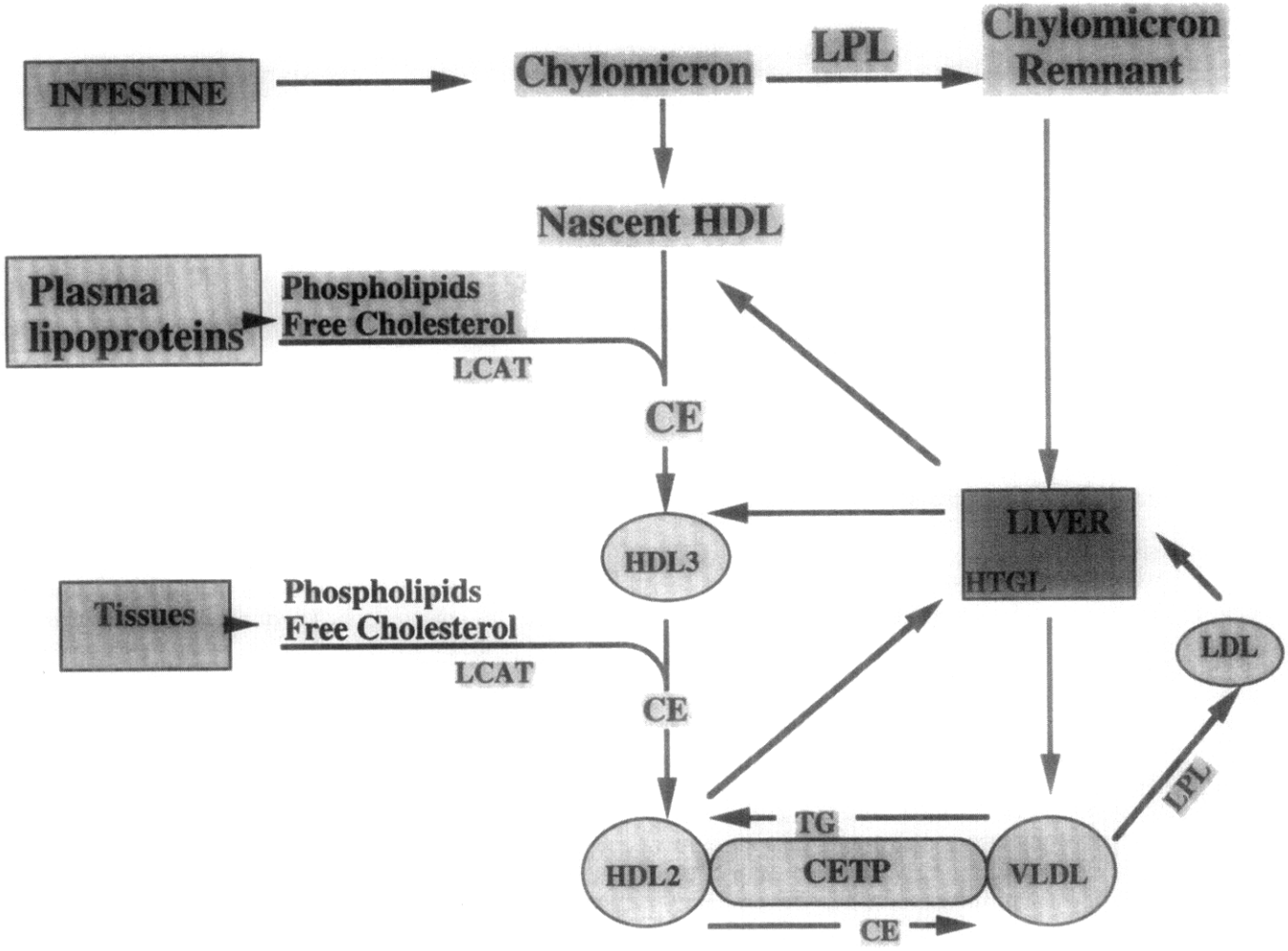

Figure 2 Overview of lipoprotein metabolism in humans. CE = cholesterol ester; TG = triglycerides; LPL = lipoprotein lipase; HTGL = hepatic triglyceride lipase; LCAT = lecithin:cholesterol acyl transferase; CETP = cholesterol ester transfer protein.

gradually enriched with cholesterol esters through the action of lecithin:cholesterol acyl transferase (LCAT).[135] The substrates for the LCAT reaction are unesterified cholesterol and phospholipids, particularly, lecithin (phosphatidyl choline). These substrates are normal components of cellular membranes and plasma lipoproteins, and arise after the cells die or after the hydrolysis of the lipoproteins. LCAT is associated with the HDL particles; therefore, the LCAT reaction occurs on the surface of the HDL particles. With the enrichment of HDL with cholesterol esters, they become more spherical, larger, and more buoyant.

HDL particles serve as substrates for the reaction that is catalyzed by cholesterol ester transfer protein (CETP), where HDL donate cholesterol ester to VLDL and IDL in exchange for triglycerides (hetero-exchange) or cholesterol ester (homo-exchange) from these lipoproteins (*Figure 2*). The triglycerides will be removed from the HDL particles when they pass through the liver through the action of hepatic triglyceride lipase (HTGL). The particles that exit from the liver have a decreased neutral lipid content, are smaller and denser, and can accept more cholesterol ester. This scheme for the metabolism of cholesterol, which is mostly mediated by HDL particles, is referred to as reverse cholesterol transport (RCT).

RCT is the term used to describe a pathway by which cholesterol in extrahepatic tissues is transported via the plasma to the liver from which it may be recycled to extrahepatic tissues or excreted into the intestine in bile (*Figure 3A*). Because it is the only pathway by which cholesterol from extrahepatic tissues is eliminated, RCT is crucial for the maintenance of the structure and function of most cells in the body, and may be important in reducing the susceptibility to atherosclerosis. Thus, alterations in any or all of the steps of RCT may predispose individuals to a higher risk for atherosclerosis.

There are several changes in HDL metabolism in patients with NIDDM. Although the precise causes are not fully known, these changes may be due to: (a) decreased synthesis of HDL; (b) impairments in RCT; or (c) both. The potential mechanisms that underlie the alterations in HDL may be related to the resistance of tissues to insulin action in NIDDM, which, in turn may influence the levels or the activity of the enzymes of HDL metabolism.

Numerous studies reported decreases in HDL cholesterol in diabetic patients.[3,4,10,63,74,136–142] Evidence shows that there is an inverse relation between plasma insulin levels and HDL cholesterol: the higher the insulin concentration, the lower the HDL.[136,138,142] This inverse relation was dem-

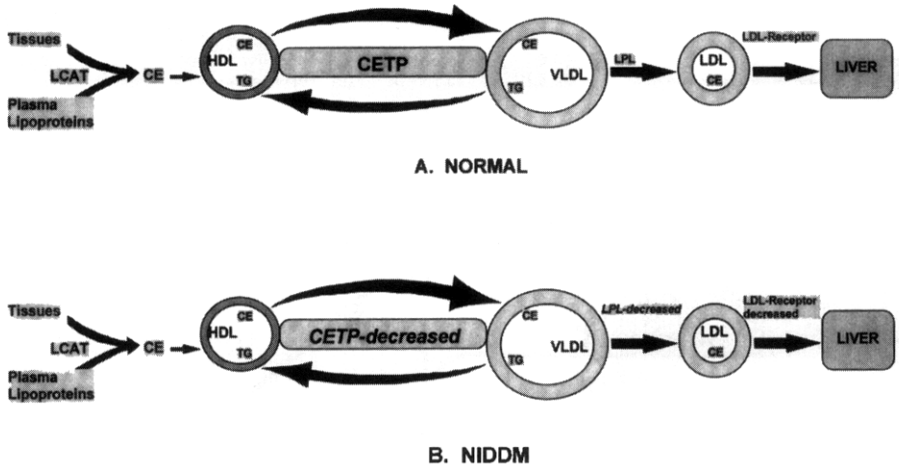

Figure 3 Reverse cholesterol transport (RCT) in normal (A) and diabetic (B) humans. CE = cholesterol ester; TG = triglycerides; LPL = lipoprotein lipase; LCAT = lecithin:cholesterol acyl transferase; CETP = cholesterol ester transfer protein.

onstrated in patients with impaired glucose tolerance[138] as well as in subjects with normal glucose tolerance.[143–145] In kinetic studies of the turnover of HDL in non-diabetic and diabetic subjects, work from Reaven's laboratory showed that the higher the plasma insulin concentration, the greater the fractional catabolic rate of HDL and the lower the HDL concentration.[146] Because high plasma insulin concentration in diabetics is more of a compensatory mechanism for the increased resistance of tissues to insulin action, it is likely that the decrease in HDL that accompanies the increase in plasma insulin levels may be more a consequence of insulin resistance rather than a result of hyperinsulinemia. This postulate is supported by evidence from our laboratory. We measured plasma HDL in a group of morbidly obese patients with or without NIDDM before and after gastric bypass surgery.[147] Fasting plasma insulin levels were high in both groups before the surgical procedure, but decreased after surgery from 21.8 to 13.5 μU/mL in the obese normoglycemic group and from 39.3 to 15.3 μU/mL in the NIDDM group. HDL cholesterol was increased by 56% in the NIDDM patients but only increased by 4% in the obese nondiabetic patients. We postulated that the improvement in insulin sensitivity, which occurs after gastric bypass surgery, may be important in influencing HDL cholesterol. A possible explanation of the variation in HDL concentration in NIDDM may be related to the decrease in LPL activity in these patients. As noted, LPL hydrolyzes triglycerides from the circulating lipoproteins and these delipidated lipoproteins are a source of HDL. Impairments in LPL function, therefore, will limit the supply of HDL, and conversely, improvement in LPL will increase this supply. These change may occur in the resistant state and after improvement in insulin sensitivity because the expression of LPL has been shown to be dependent on insulin.

Improvement in insulin sensitivity, in addition to raising plasma HDL cholesterol concentration, appears to favorably affect the subpopulation distribution of HDL. We reported differences in the composition of HDL of morbidly obese patients with or without diabetes compared with lean, nondiabetic control subjects.[147] The compositional analysis showed an increase in the protein, but a decrease in the cholesterol ester content, suggesting that HDL of these patients may be composed mostly of the denser, less buoyant fraction of HDL (HDL_3). Analysis of the subpopulation distribution of HDL showed that HDL_2 mass was lower in the patient group but HDL_3 higher. After gastric bypass surgery, which increases insulin sensitivity, HDL composition became more nearly like that of the lean controls, with the percentage of protein decreased and that of cholesterol ester increased compared with presurgery data. Accompanying these changes in the composition was an increase in the levels of HDL_2 and a decrease in the average hydrated density of plasma HDL. These changes, which were more pronounced in the obese diabetic patients than the obese normoglycemic subjects, may be related to the function of hepatic triglyceride lipase (HTGL).

The function of HTGL is similar to that of LPL in that it hydrolyzes the triglycerides of triglyceride-rich lipoproteins. In addition, HTGL hydrolyzes the triglycerides of HDL when they pass through the liver. As a result, HDL get smaller and revert to the more dense HDL_3 subfraction (*Figure 2*). Thus, as is the case with LPL, HTGL is important in the metabolism of both triglyceride-rich and cholesterol-rich lipoproteins. This implies that aberration in the function of HTGL will directly or indirectly impact the metabolism of the lipoproteins and may contribute to cardiovascular risk and incidence. What, if any, are the alterations in HTGL function in NIDDM? What are the consequences of these alterations on lipoprotein function?

Early demonstration of the association of NIDDM and aberrant HTGL function came from a study by Nikkila et al.[148] in which diabetic patients (both type I and II) were matched on age and sex with nondiabetic controls. They found that HTGL activity in postheparin plasma was similar in diabetics and controls, with the exception of hypertriglyceridemic NIDDM subjects who had higher HTGL activity than the corresponding controls. Studies by Baynes et al.[149] showed that HTGL activity correlated negatively with the insulin sensitivity index, and postively with plasma insulin concentrations. Patients with NIDDM are usually hyperinsulinemic and insulin-resistant and, thus, HTGL would be expected to be elevated in such patients. To date, few

studies have appeared that examined the direct effects of insulin on the expression of HTGL. Nonetheless, the generally accepted view is that HTGL activity is increased in patients with NIDDM, whereas LPL activity is decreased.

Aberrations in HTGL are associated with increased coronary heart disease. Studies by Kuusi et al.[125] showed HTGL activity was higher in subjects with low HDL values compared with matched subjects with high HDL levels. Furthermore, there is evidence that shows that the more antiatherogenic HDL_2 subfraction is negatively correlated with HTGL activity. In addition to the associations of altered HTGL activity with HDL levels and subpopulation distribution, there is also experimental evidence that implicates HTGL in the compositional alterations in LDL. Studies by Zambon et al.[150] reported a relationship between HTGL and LDL composition in patients with coronary heart disease. They also reported a significant negative correlation between HTGL activity and LDL particle size and concluded that HTGL was influential in determining LDL phenotype. Consistent with this observation, Auwerx et al.[151] reported that in HTGL deficient patients, LDL was larger and more buoyant than LDL of control subjects. These findings suggest that alterations in HTGL influence the subpopulation distribution of both LDL and HDL. Increased activity of HTGL, which has been reported in patients with NIDDM, favors the formation of small LDL and HDL particles, both of which have been implicated in early atherogenesis.

The changes in HDL concentration in diabetics may also be related to changes in RCT. When considering the effects of NIDDM on RCT, the changes in the major four steps of RCT need to be addressed. The steps in RCT, shown in *Figure 3A* are:

1. Efflux of cholesterol from cell membranes to HDL in the extracellular space
2. Esterification of HDL-cholesterol by LCAT
3. Transfer of cholesterol ester from HDL to other lipoproteins by CETP
4. Delivery of the cholesterol ester to the liver

Little information describing the effects of diabetes on cholesterol efflux from cells is available. Thus, this is a fertile area of future research. More information is available on the effects of NIDDM on the function of LCAT and CETP.

As described earlier, LCAT and CETP are two plasma enzymes that are involved in the metabolism of cholesterol in the plasma (*Figure 2*). LCAT facilitates the synthesis of cholesterol esters from the free cholesterol and fatty acids that are shed by peripheral tissue. With respect to the effects of NIDDM on LCAT function (*Figure 3B*), there is evidence that shows that LCAT is either unchanged[152,153] or decreased[154,156] in these patients. It appears that the differences in the findings may be the result of differences in the patient populations studied. In well-controlled NIDDM, it appears that LCAT activity was not different from controls, whereas in uncontrolled diabetes, LCAT activity appears to be deficient. This decrease may explain the reported decrease in HDL_2 that has been observed in diabetic patients. Furthermore, it seems that LCAT activity is modulated by insulin, whose effects on LCAT expression need to be investigated further.

Human CETP has been purified, sequenced, and cloned.[157–162] Recently, it was shown that a deficiency in CETP resulted in high plasma HDL concentrations.[163–165] This enzyme has been shown to be regulated by the composition of the plasma lipoproteins,[166–168] dietary cholesterol[169–171] and by several cholesterol-lowering drugs.[172–174] However, the direct effects of insulin on the regulation of the expression of the enzyme have not been explored. Indirect evidence of the involvement of insulin in modulating CETP function comes from studies of patients with IDDM.[175]

The findings with respect to CETP function in NIDDM (*Figure 3B*) and its regulation by insulin are not consistent. Initial findings from Fielding's laboratory[176] showed that cholesterol ester transfer from HDL to LDL was inhibited in poorly controlled NIDDM, and insulin therapy normalized the hyperglycemia and cholesterol metabolism. More recently, Kahari et al.[177] showed that NIDDM patients with or without cardiovascular disease had decreased plasma CETP activity. Data from our laboratory[178] showed that CETP activity in the plasma and adipose tissue of morbidly obese patients with NIDDM was lower than that of morbidly obese patients without NIDDM and lean controls. Interestingly, after improvement in insulin sensitivity, the activity of CETP in the NIDDM patients returned to more normal values. Bagdade and coworkers, on the other hand,[179] reported an increase in cholesterol ester transfer in four patients with NIDDM compared with four nondiabetic controls. Taken together, these results suggest that insulin has a role in CETP function, and that CETP function may be altered in NIDDM.

That insulin has a role in modulating CETP function came from studies in the hamster, an animal model that exhibits CETP activity in adipose tissue, muscle, and liver.[180,181] In vitro studies with cultured adipocytes or adipose tissue pieces, showed that CETP activity was increased in the culture medium, but decreased in the tissue preparations.[181] In patients with NIDDM, on the other hand, Sutherland et al.[182] observed an inhibitory effect of a 2-hour hyperinsulinemic euglycemic clamp on CETP activity in the plasma. Preliminary results from our laboratory confirmed these observations.[178] Importantly, we also observed an increase in CETP activity only in NIDDM patients whose insulin sensitivity improved after gastric bypass surgery, but not in those who remained resistant to insulin action.[178] These data suggest that insulin stimulates expression of CETP as long as the tissues are responsive to insulin, but that its effects are partially or wholly negated if the tissue is resistant to insulin action. Clearly, the role of insulin in modulating CETP expression needs to be investigated further.

As for the fourth step in RCT, namely, the delivery of cholesterol to the liver, there is extensive evidence that shows that the LDL receptor-mediated pathway may be deficient in diabetics. This may be due, in part, to the role of insulin in modulating the expression of the LDL receptors. Because the tissues of diabetic patients, including liver, are resistant to insulin action, it is highly likely that delivery of cholesterol to the liver may be impaired. Clearly, this issue needs to be explored in more detail in future research efforts.

In summary, the major changes in HDL in NIDDM are (a) decreased plasma HDL-cholesterol levels and (b) a shift in the subpopulation of HDL particles toward the smaller, cholesterol ester-poor HDL subfraction, namely HDL_3. These changes are most likely due to decreased synthesis of HDL as well as to aberrations in reverse cholesterol transport. The underlying cause of these alterations may be the resistance of tissues to insulin action that, in turn, affects the functions of critical enzymes in the tissues that metabolize HDL.

IV. Summary and conclusions

Although the risk and incidence of coronary heart disease in diabetic patients are higher than in the nondiabetic population, the precise mechanisms underlying CHD are not fully known. Several aberrations in the metabolism of plasma lipoproteins that may contribute to CHD in diabetes have been identified. Despite the extensive research in lipoprotein metabolism for the past three decades, there are still many areas that need to be explored and defined further. One of the major reasons behind the slow pace of discovery is the lack of an experimental animal model that simulates human NIDDM. In humans, NIDDM is a syndrome that is characterized by insulin resistance, which is accompanied by hyperglycemia and hyperinsulinemia. All of these anomalies contribute to aberrations in lipoprotein metabolism, and efforts have been directed at determining the effects of each of these characteristics, alone or in combination, on the metabolism of the lipoproteins. Thus, there are some conflicting results that reflect the heterogeneity of diabetes, and the populations studied. Despite all these drawbacks, the progress that has been made in the last decade has yielded an impressive data base that has helped in finding better treatments for the control of diabetes, and which will, eventually, lead to the cure of this syndrome.

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