Mechanism of Action of Niacin on Lipoprotein Metabolism

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Current Atherosclerosis Reports 2000, **2:**36–46
Current Science Inc. ISSN 1523–3804

It is generally accepted that the increased concentrations of apolipoprotein (apo) B containing very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), and decreased levels of apo AI containing high-density lipoproteins (HDL) are correlated to atherosclerotic cardiovascular disease. Current evidence indicates that the post-translational apo-B degradative processes regulate the hepatic assembly and secretion of VLDL and the subsequent generation of LDL particles. The availability of triglycerides (TG) for the addition to apo B during intracellular processing appears to play a central role in targeting apo B for either intracellular degradation or assembly and secretion as VLDL particles. Based on the availability of TG, the liver secretes either dense TG-poor VLDL2 or large TG-rich VLDL1 particles, and these particles serve as precursors for the formation of more buoyant or small, dense LDL particles by lipid transfer protein- and hepatic lipase-mediated processes. HDLs are a heterogenous class of lipoproteins, and apo AI (the major protein of HDL) participates in reverse cholesterol transport, a process by which excess cholesterol is eliminated. Recent studies indicate that HDL particles containing only apo A-I (LPA-I) are more effective in reverse cholesterol transport and more anti-atherogenic than HDL particles containing both apo A-I and apo A-II (LPA-I + A-II).

Introduction

Niacin is an effective, unique lipid-regulating agent that reduces total plasma cholesterol, apoliprotein (apo) B, triglyceride (TG), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) and increases high-density lipoprotein (HDL) levels. It also decreases small, dense LDL particles, lipoprotein(a), and selectively increases LPA-I particles. Several clinical trials indicated that treatment with niacin significantly reduced total mortality, coronary events, and retarded the progression and induced the regression of coronary atherosclerosis. Using human hepatoblastoma cells (Hep G2), recently we have provided new conceptual insight on the mechanisms of action of niacin to modulate lipoprotein metabolic processes. These studies indicated that niacin inhibited TG synthesis and increased intracellular apo-B degradation in hepatocytes. Niacin-mediated inhibition of TG synthesis, by inhibiting apo-B translocation through the endoplasmic reticulum (ER) membrane, may increase intracellular apo-B degradation resulting in decreased apo-B secretion. This novel mechanism would support the clinical observations of niacin-mediated decreased VLDL–TG transport and VLDL levels, and the generation of more buoyant LDL particles in patients with hyperlipidemia. Additional studies examining apo AI and HDL metabolic events indicated that niacin specifically increases apo A-I by decreasing hepatocellular uptake of HDL-apo AI without affecting HDL-cholesteryl ester uptake. Recent evidence indicates that niacin inhibits hepatic LPA-I uptake more than LPA-I+AII particles. This novel mechanism results in increased apo A-I concentrations, resulting in improved efficiency of reverse cholesterol transport. More mechanistic research may not only lead to new drug discovery, but form the rationale for combination therapy using agents with different mechanisms of action to both decrease VLDL/LDL and increase HDL levels.

Abnormalities in lipids and lipoprotein metabolism, including mainly VLDL, LDL, and HDL, are involved in atherosclerotic cardiovascular disease (ASCVD). During the past two decades, a large body of clinical and basic science evidence has conclusively indicated that the alterations in plasma lipids (*eg*, cholesterol, TG) and lipoproteins are primary causative risk factors in the development of coronary artery disease (CAD) [1•]. In a Multiple Risk Factor Intervention Trial (MRFIT), a curvilinear relation between total cholesterol and CAD mortality was clearly demonstrated in a large number of patients screened for more than 6 years [2]. Numerous human clinical trials using quantitative angiographic techniques have assessed the impact of lipid-regulating treatment in the progression of CAD [3]. The beneficial effects of lowering plasma cholesterol and particularly LDL-cholesterol to decrease or even prevent CAD progression and even induce CAD regression in patients with hyperlipidemia have been unequivocally demonstrated in both primary and secondary prevention trials [1,4]. In addition to cholesterol and LDL levels, the

plasma levels of TG and TG-containing lipoproteins (*ie*, VLDL) are also shown to be positively related to the development of CAD. Some epidemiologic trials, such as the Prospective Cardiovascular Munster (PROCAM) and Framingham studies, have indicated that increased plasma TG is an independent risk factor for CAD [1]. In the Monitored Atherosclerosis Regression Study (MARS), plasma TG and the ratio of total cholesterol/HDL cholesterol were correlated to the lesion progression in mild-to-moderate atherosclerotic lesions.

Contrary to the positive pathobiologic role of VLDL and LDL, epidemiologic studies have shown that the plasma concentrations of HDL bear an inverse relationship to atherosclerotic CAD. Several epidemiologic studies have indicated that the decreased levels of HDL are correlated to the progression of CAD [1,5•]. Other prospective studies confirmed these observations and demonstrated that the amount of HDL-cholesterol as a fraction of total cholesterol was an important inverse determinant of CHD risk [5•]. Recent studies have shown that the intervention made to increase HDL levels regressed cardiovascular events, suggesting clearly the antiatherogenic properties of HDL [1,5•].

Because of the pathobiologic relationship between lipids/lipoprotein metabolism and ASCVD, it is very important to understand the intricate cellular and molecular processes involved in lipid and lipoprotein metabolic pathways (*ie*, synthetic and catabolic events) that govern plasma levels of lipids such as cholesterol and TG, and lipoproteins (*eg*, VLDL, LDL, HDL). In this article, we briefly review the metabolism of lipoproteins and their relationship to atherogenic processes. We believe that a background review of current status of the physiology, biochemistry, and hepatocellular regulation of lipoproteins is necessary to fully appreciate the significance of recent concepts on the pharmacologic biochemistry of niacin.

Regulatory Processes in Very Low-density Lipoprotein and Low-density Lipoprotein Metabolism

Plasma lipoproteins transport hydrophobic endogenous molecules, including cholesterol, TG, and phospholipid, through the aqueous plasma compartment to sites of utilization or catabolism. LDL particles are the major carriers of plasma cholesterol, whereas the VLDL particles transport endogenous TG. Liver is the major organ for the production and secretion of human plasma VLDL particles. However, the intestine also produces lipoproteins similar in size and lipid composition to the lower molecular weight truncated form of apolipoprotein (apo) B [6]. The secreted VLDL particles, through extrahepatic lipoprotein lipase-mediated TG hydrolysis, are converted to form partially TG-depleted intermediate-density lipoprotein (IDL) particles relatively enriched in cholesterol. Kinetic studies examining the fate of radiolabeled-VLDL in humans indicated a precursor-product relationship in the apo B of VLDL and IDL [7]. Subsequently, plasma LDL particles are derived from IDL and VLDL particles. The formation of LDL from VLDL in the circulation is regulated by complex cellular processes that are mediated by various lipid-regulating enzymes and lipid transfer proteins.

In the circulation, VLDL particles are the precursors of LDL particles, and it has been recognized that the overproduction of VLDL results in hyperlipidemia and increased LDL levels in a large percentage of patients [8]. The central mechanism in the formation and secretion of VLDL particles appears to be the hepatic intracellular processing of apo B and its association with lipids in the assembly of VLDL particles targeting either for secretion or degradation. During the past decade, this area of research has been under extensive investigation to understand the regulatory processes involved in apo B intracellular processing, and VLDL assembly and secretion. In this section of the review, we have briefly described: 1) the current understanding on the regulatory mechanistic processes involved in apo B post-translation degradative or secretory events, 2) the impact of secreted VLDL particle composition on the formation of buoyant or dense LDL particles with differing atherogenicity, 3) the pathobiologic role of LDL particles in atherogenesis, and 4) the biochemical pharmacology and mechanisms of action of niacin in apo B post-translational processing and VLDL particle secretion.

Current understanding of the regulatory mechanistic processes involved in apolipoprotein B post-translational degradative and secretory events

Apo B 100 (apo B), the major protein of VLDL and LDL, is essential for the assembly and secretion of VLDL particles by hepatocytes. Human apo B100 is a large hydrophobic protein with a molecular weight of about 520 kD, which is synthesized in the polysomes bound to the rough ER of the liver. Regulation of the assembly and secretion of apo B-containing lipoproteins (*eg*, VLDL) from hepatocytes involves complex cellular processes, including the events related to the localization of newly synthesized apo B as it translocates across the ER, the post-translational apo B degradation, and the mechanisms governing the synthesis and addition of core lipids to the nascent VLDL particle for secretion [9,10]. It is important to note that the synthesis of apo B and apo B mRNA levels generally do not alter significantly under experimental conditions in which apo B and VLDL secretion is altered several fold [10]. Considerable evidence indicates that a large amount of de novo synthesized apo B is not secreted, but rather is post-translationally degraded in hepatocytes. Further studies indicated that apo B is synthesized on the rough ER and then translocated from the ER membrane to the lumen for rapid secretion from the hepatocytes [10–13]. It has been suggested that the critical event appears to be the prolonged association of apo B with the ER membrane to target apo B for degradation, in contrast with the rapid translocation facilitating secretory pathway [10]. The intracellular post-translational processing of apo B and its deg-

radation are mainly regulated by the protease-mediated degradation, the synthesis and the availability of lipids, and the transfer of lipids by microsomal triglyceride transfer protein (MTP)-mediated events. For example, the proteases localized either in the cytosol or on the cytosolic side of ER membrane are implicated in apo-B degradation prior to secretion. Using cysteine active site protease inhibitor (N-acetyl-leucyl-leucyl-norleucinal; ALLN, an agent shown to block the degradation of translocation-arrested apo B), Bonnardel and Davis [14] have shown that the translocation, but not degradation, determines the intracellular fate of de novo synthesized apo B.

The current evidence indicates that the rate of lipid synthesis and the availability to lipidate apo B play a critical role in the translocation of apo B across the ER membrane, resulting in either secretion or intracellular degradation prior to secretion. For example, oleic acid (which increases TG synthesis and secretion) has been shown to stimulate apo B secretion from Hep G2 cells [10,15] by facilitating the translocation of newly synthesized apo B away from proteases [16] that are associated with the ER, and thereby protecting newly synthesized apo B from intracellular degradation. Supporting evidence for the participation of TG in apo B degradation/secretion was provided by demonstrating that the inhibition of fatty acid and TG synthesis inhibited apo-B secretion [17]. Furthermore, addition of oleic acid to the medium of ALLN-treated Hep G2 cells increased the secretion of apo B to a greater extent than in Hep G2 cells not treated with ALLN [16], suggesting that oleic acid increased the translocation of apo B. The finding that oleic acid but not ALLN increases the translocation of apo B suggest that lipids, not the amount of apo B, signals an increase in apo-B translocation and secretion. Although the availability of TG has been shown to be essential for apo-B translocation and secretion, the role of cholesterol and cholesterol esters in apo-B degradation is controversial, and demonstrated both the requirement and nonrequirement of cholesterol/cholesterol esters in apo-B degradation in Hep G2 cells and hamster hepatocytes [10,17–19]. Furthermore, MTP, a lipid carrier protein within the membrane, it has been suggested, plays an important role in facilitating the secretion of apo B-containing lipoproteins. Mutations in the gene encoding for the 97-kD subunit of MTP have been shown in a complete lack of the secretion of apo B-containing lipoproteins in rare autosomal recessive abetalipoproteinemia [10]. Inhibition of cellular MTP inhibited apo-B secretion from Hep G2 cells [20], suggests clearly the requirement MTP in apo-B secretion.

Based on these observations, it has been suggested that the newly synthesized apo B after partial cotranslational translocation through the ER membrane has the potential either for complete translocation and secretion or for degradation. The higher the duration of apo B localized in this partially translocated state, the higher the probability that nascent apo B will be degraded by proteases. Under lipid-poor conditions (*eg,* inhibition of TG synthesis), the large majority of newly synthesized apo B remains partially translocated long enough for efficient degradation to occur. However, under lipid-rich conditions (*eg,* fatty acid mobilization in vivo or oleate addition experimentally in vitro), the apo B interacts with regions of the ER where active lipid synthesis occurs, leading to reduced apo B degradation (and increased secretion) by accelerating its translocation from the protease-containing compartment. Thus, alterations in one or more of these hepatic intracellular processes (*eg,* protease-mediated apo-B degradation, lipid synthesis, the availability of lipids for the addition to apo B, MTP-mediated lipid transfer to apo B, and translocation of apo B across the ER) would profoundly influence the VLDL assembly and secretion by hepatocytes, which in turn regulate the levels of VLDL and LDL.

Lipid composition and size of secreted very low-density lipoprotein particles in the formation of large buoyant or small, dense low-density lipoprotein atherogenic particles

In addition to the VLDL assembly and secretion, the regulatory role of lipid synthesis and lipid availability for addition to apo B polypeptide during its secretion has been proposed to play a pivotal role in the formation of heterogenic LDL particles, such as more buoyant native LDL or small, dense LDL particles. To address the clinical relevance of heterogeneity in LDL particles, several clinical studies were undertaken to examine their association to atherosclerotic CAD [21]. In case-control studies, the predominance of small, dense LDL particles (mainly LDL-III with density 1.044–1.06), designated as LDL subclass pattern B, has been shown to be associated with increased CHD risk in myocardial infarction and angiographically defined coronary disease [21]. A much stronger association of small, dense LDL and CHD risk is drawn from prospective or longitudinal studies in which changes in disease status are correlated with variations in LDL subparticles [21].

Although the precise mechanisms for the formation of small, dense LDL particles are not completely understood, both genetic (in approximately 30% of the patient population) and metabolic (in approximately 70%) abnormalities are shown to participate in the generation of small, dense LDL particles [21]. Current evidence indicates that the metabolic conditions associated with hypertriglyceridemia (*eg,* adiposity, insulin resistance) are commonly associated with increased small, dense LDL particles, suggesting that the overproduction or impaired clearance of triglyceride-rich VLDL particles may play an important role in the generation of small, dense LDL particles [21]. Additionally, it has been suggested that the VLDL assembly in the liver and regulatory events of addition of TG to the VLDL particles play a central role in the formation of small dense LDL particles. As discussed earlier, the VLDL assembly in hepatocytes is regulated by complex processes including the availability of lipids such as TG and lipoprotein assembly initiation by MTP-mediated addition of an initial lipid load to the growing apo B-polypeptide chain to form a nascent particle [22••]. The

bulk of lipid is added in a second step that leads to the synthesis and secretion of lipoproteins ranging in size from very large triglyceride-rich VLDL1 particles (Sf 60–400) to a denser cholesteryl ester-rich VLDL2 particles (Sf 20–60). Evidence suggests that the releases of these VLDL species can be regulated independently of one another, and have differing metabolic fates in the circulation. For example, it has been proposed that apo B100 acquires neutral lipid by MTP-mediated events as it translocates in the rough ER leading to the secretion of VLDL2 particles. In hypertriglyceridemic conditions or in situations of increased availability of triglycerides in hepatocytes, the additional bulk of triglycerides is added to the nascent lipoprotein particles leading to the formation and secretion of triglyceride-rich large VLDL1 particles. The cholesteryl ester transfer protein (CETP) mediates triglyceride movement from VLDL1 to LDL particles (or possibly IDL), generating TG-rich LDL particles. This promotes the hydrolysis of the LDL triglyceride core by hepatic lipase generating small, dense LDL particles. In the absence of high TG availability, LDL-TG is not a preferred substrate for hepatic lipase, and LDL remains larger and more buoyant. Thus, the synthesis and the availability of TG and its addition to apo B during post-translational apo B processing appears to have an important role in generating VLDL particles with varied TG content and size, and the subsequent formation of more buoyant or small, dense LDL particles. Alterations in TG synthesis and its availability for the addition to apo B during VLDL assembly would be critical in the generation of more buoyant or small, dense LDL particles with differential atherogenicity.

The pathobiologic role of low-density lipoprotein particles in atherogenesis

Atherosclerotic vascular disease is a fibroproliferative disease characterized by vascular endothelial dysfunction and activation, smooth muscle cell hypercellularity and intimal migration, accumulation of lipid-laden foam cells, and excessive deposition of extracellular matrix proteins including collagen, elastic fibers, and proteoglycans. Currently, major emphasis has been placed on understanding the mechanisms by which LDL particles participate in various pathobiologic processes involved in the development of atherosclerosis. The novel discovery of LDL-receptor pathway by Goldstein and Brown [23] in various cell types and the demonstration of nonsaturable distinct scavenger or acetyl-LDL receptors in macrophages provided an initial pathobiologic role for atherogenic lipoproteins in the transformation of monocyte-macrophages into lipid-laden foam cells of atherosclerotic lesions. Additional significant discoveries made by Steinberg *et al*. [24] have shown that either circulating LDL or LDL entering the arterial wall may undergo oxidative modification by interacting with vascular cells or circulating cells, thus altering its catabolic properties to be taken up by the scavenger receptors on macrophages and provoking the formation of lipid-laden foam cells. Ross and associates proposed the "Response-to-Injury" hypothesis to highlight the pathobiologic role of hyperlipidemia in endothelial activation processes [25]. These investigators demonstrated an increased adherence of circulating monocytes to the arterial endothelium in cholesterol-fed animal models, suggesting that hypercholesterolemia or atherogenic lipoproteins may activate the endothelium to produce various new cytokines and growth factors. Subsequent studies using in-vitro and in-vivo model systems indicated that oxidatively modified LDL (minimally and oxidized LDL or its components) can activate endothelial cell expression of various adhesion molecules, monocyte chemoattractants, and growth factors involved in endothelial migration and accumulation of monocytes and cell proliferation [26–30]. Additionally, atherogenic lipoproteins (*eg*, LDL and its oxidized variants) are also shown to downregulate endothelial nitric oxide synthase resulting in decreased vascular nitric oxide (vasodilator) levels and increased endothelin (vasoconstrictor) concentrations, which are known characteristic features of endothelial dysfunction [1,31].

In addition to the positive correlation of small dense LDL and coronary artery disease, recent studies provided the cellular mechanisms by which these LDL particles stimulate atherogenic events. The pathobiologic processes modulated by small, dense LDL include slower removal from the circulation, increased susceptibility to oxidation, greater affinity to interact with arterial wall proteoglycans, greater capacity to promote enrichment of aortic endothelial cells with cholesteryl esters, increased stimulation of thromboxane synthesis by endothelial cells, and increased generation of intracellular free calcium in aortic smooth muscle cells [21]. Thus, these studies provide significant evidence to suggest that LDL particles can serve as a pathobiologic stimulus for the activation and dysfunction of endothelial wall to produce various cytokines and growth factors involved in monocyte infiltration into the subendothelium, formation of foam cells, smooth muscle cell proliferation and migration to the intimal region, which are primary characteristic features of atherosclerosis.

The effect of niacin on lipid metabolism and on the molecular mechanisms of action in intracellular apo B degradation and triglyceride synthesis

Niacin has been widely used clinically to regulate abnormalities in plasma lipid metabolism and in the treatment of atherosclerotic cardiovascular disease. In pharmacologic doses, niacin reduces total plasma cholesterol, triglycerides, VLDL, LDL, and LP(a), and increases HDL and certain important components and subfractions [1,5•]. Furthermore, various clinical trials including Coronary Drug Project, Cholesterol Lowering Atherosclerosis Study and Familial Atherosclerosis Treatment Study indicated that treatment with niacin significantly reduced total mortality, coronary events, and retarded the progression and induced regression of coronary atherosclerosis [1,5•]. Recent studies from the Familial Atherosclerosis Treatment Study indicated that the treatment with niacin significantly increased buoy-

Table 1. In vivo plasma turnover and in vitro hepatocellular mechanisms of action of niacin on apo B, VLDL, LDL, and apo AI and HDL metabolic processes

Apo B, VLDL, and LDL Metabolic Mechanisms	Reference	Apo AI and HDL Metabolic Mechanisms	Reference
In-Vivo Studies in Humans		In-Vivo Studies in Humans	
Decreased production of VLDL	Grundy SM *et al.* [33]	Decreased Apo AI fractional catabolic rate	Blum CB, et al. [61]; Shephard J, *et al.* [62]
In-Vitro Studies in Hepatocytes		In-Vitro Studies in Hepatocytes	
Increased Apo B degradation	Jin FY, *et al.* [36••]	Increased accumulation of Apo AI in culture media	Jin FY, *et al.* [63••]
Decreased Apo B secretion	Jin FY, *et al.* [36••]	No change in Apo AI de novo synthesis	Jin FY, *et al.* [63••]
Decreased fatty acid and TG synthesis	Jin FY, *et al.* [36••]	Decreased HDL-apo AI uptake/ removal	Jin FY, *et al.* [63••]
Decreased fatty acid release from adipocytes	Carlson LA [34]; Lee HM, *et al.* [35]	No change in HDL-cholesteryl ester uptake/removal	Jin FY, *et al.* [63••]
No change in Apo B de novo synthesis	Jin FY, *et al.* [36••]	Increased ability of secreted Apo AI particles to efflux cholesterol by fibroblasts	Jin FY, *et al.* [63••]
No change in Apo B uptake [36]	Jin FY, *et al.* [36••]		

Apo—apolipoprotein; HDL—high-density lipoprotein; LDL—low-density lipoprotein; VLDL—very low-density lipoprotein.

ant LDL (10%) and decreased hepatic lipase (17%) in patients with elevated apo B levels and documented coronary disease [32•]. Using multivariate analysis, these investigators have shown that the increase in LDL buoyancy was strongly associated with CAD regression, accounting for 37% of the variance of changes in coronary stenosis, followed by reduction in apo B (5% of variance). It was suggested that lipid-lowering therapy-associated changes in LDL buoyancy may favorably influence CAD progression. Earlier in-vivo studies in humans by Grundy *et al.* [33] demonstrated that the treatment with niacin reduced VLDL–TG synthetic rate (transport) as determined by multicompartmental kinetic analysis following the injection of radiolabeled glycerol as a precursor (Table 1). Additionally in early studies, it has been suggested that the observed reduced VLDL–TG in niacin-treated patients may be a result of inhibition in release of free fatty acids by adipose tissue (Table 1) [34,35]. Till recently, decreased adipose tissue lipolysis and decreased fatty acid mobilization have been regarded as the major mechanisms of action of niacin.

In order to gain insight into the mechanism of action of niacin on VLDL/LDL metabolism, we have recently examined the effect of niacin on regulatory processes involved in apo B degradation and secretion in human hepatoblastoma cells (Hep G2). The results of these studies showed that niacin (0.5–3 mM), in a dose-dependent fashion, increased apo B intracellular degradation and decreased subsequent secretion of apo B into the culture media (Table 1) [36••]. Additional studies were done to examine the rate of apo B degradation by chasing at various time points (0–180 min). The data from pulse–chase studies (pulse, 15 min; chase, 0–180 min) indicated that niacin significantly increased the total degradation of apo B during chase periods of 20 min to 180 min (Table 1) [36]. Additionally, niacin had no effect on the steady-state mRNA expression of apo B, and niacin did not alter the uptake of LDL by Hep G2 cells (as measured by the uptake of 125I-LDL). These data clearly suggest that niacin did not affect either the synthesis or uptake of apo B/LDL in Hep G2 cells. Thus, the observed effects of niacin on apo B degradation would be mainly through post-translational processing events. Because protease-mediated degradation of apo B and MTP-mediated transfer of lipids to apo B are important regulatory events in apo B degradation and secretion, we examined whether niacin had any direct effect on MTP activity or protease-mediated apo-B degradation (Table 1). Niacin did not alter MTP activity when compared with controls. Studies assessing the effects of niacin on protease-mediated apo B degradation were performed using ALLN, a protease inhibitor. Similar to the earlier studies, treatment of Hep G2 cells with ALLN significantly decreased apo-B degradation when compared with control cells. However, the apo-B degradation in ALLN and ALLN plus niacin treated cells was not significantly different. These results suggest that the effect of niacin to induce apo-B degradation is independent of ALLN-inhibitable protease-mediated pathways.

Additional studies were performed to determine whether alterations in the association of newly synthesized TG with apo B is involved in niacin-induced apo-B degradation. To explore this possibility we used oleic acid (which increases TG synthesis), a known agonist that has been employed to decrease intracellular apo B degradation (and increase apo-B secretion). The incubation of niacin-pretreated cells with oleate significantly increased cellular apo-B degradation when compared with oleate treatment alone (Table 1). These data strongly suggest that niacin-induced apo-B degradation may be dependent on the path-

ways involving the synthesis and association of lipids prior to apo B processing. Examination of the direct effects of niacin on lipid synthesis indicated that niacin significantly inhibited TG production at two synthetic sites: a) fatty acid synthesis from acetate, and b) esterification of fatty acids to form TG. However, niacin did not alter the synthesis of cholesterol or cholesteryl esters or phospholipids in Hep G2 cells (Table 1). Based on these observations, we have hypothesized that niacin may be acting on key committed enzymes in fatty acid and TG synthesis (*eg*, fatty acid synthase, diacylglycerol acyltransferase).

Based on the these observations, we propose that niacin, by inhibiting key committed enzymes in fatty acid and TG synthesis, inhibits TG synthesis and mass in hepatocytes. The niacin-mediated inhibition of TG synthesis and availability may limit apo-B lipidation, leading to the delayed translocation of apo B across the ER membrane, which in turn may increase intracellular apo-B degradation. Additionally, niacin-mediated inhibition of TG synthesis and limited availability of TG may inhibit the generation of large TG-rich VLDL1 particles, which in turn inhibit the formation of small, dense LDL particles. These novel mechanistic concepts derived from studies in Hep G2 cells would support the clinical observations of niacin-mediated inhibition of VLDL–TG transport, decreased plasma VLDL, and TG levels, and decreased small, dense LDL particles in patients with hyperlipidemia. However, additional research on the effects of niacin on the key enzymes involved in TG synthesis and in primary human hepatocytes is necessary to further validate these concepts.

Regulatory Processes in High-density Lipoprotein Metabolism

As discussed earlier, HDL are inversely correlated to the development of ASCVD. HDL are a complex class of lipoproteins, comprised mainly of spherical particles with a hydrophobic core of cholesterol esters and triglycerides and an outer hydrophilic layer of apos, phospholipids, and unesterified cholesterol. The protein mass of HDL contains apo AI and apo AII as major proteins (accounting for approximately 60% and 30% of protein mass respectively) and several minor proteins, including apo CI-III, apo E, and apo AIV. The liver and intestine are major organs for the synthesis of HDL components including apos and lipids. After the synthesis and secretion of HDL components, the extracellular bilamellar particles undergo finely regulated metabolic processes mediated by lipid-metabolizing enzymes and lipid-transfer proteins leading to the formation of spherical HDL^3 and subsequently HDL^2 particles. HDL particles circulating in the plasma consist of heterogeneous population, and are classified into subfractions based on mainly physical and chemical characteristics. For example, HDL^2 (d = 1.063-1.125 g/mL) and HDL^3 (d = 1.125–1.21 g/mL) are major HDL subfractions that are separated by density ultracentrifugation. Using immunochemical methods, HDL particles are fractionated into mainly two subfractions, including apo AI-containing particles without and with apo AII (LP-AI and LP-AI + AII, respectively). Several lines of evidence suggest that these HDL subfractions have somewhat different metabolic regulation and physiologic functions and also their association to atherogenesis. In the following sections, we briefly review 1) the physiological functions of HDL particles, 2) the metabolic processes regulating the plasma concentrations of HDL and their important components, apo AI and cholesterol, and 3) mechanisms by which niacin affects HDL synthetic and catabolic events.

Functions of high-density lipoprotein

The cardioprotective effects of HDL have been largely attributed to the ability of apo AI-containing HDL particles to initiate cholesterol efflux and thereby facilitate the removal of excess cholesterol from peripheral tissues and its delivery to the liver for degradation through reverse cholesterol transport pathway (RCT). It has been indicated that LP-AI particles are more potent in effluxing cholesterol than LP-AI + AII particles in adipocytes [39], and LPA-I particles are more efficient donors of cholesteryl esters than LPA-I + A-II [40]. However, Cheung *et al.* [41] and Johnson *et al.* [42] have reported that there were no significant differences in cholesterol efflux by LP-AI or LP-A + AII in various cell types, including rat hepatoma cells, human skin fibroblasts, and rabbit aortic smooth muscle cells. In addition to the major role of HDL in RCT pathway, HDL has also been shown to have beneficial effects on various vascular and related events potentially involved in the development of atherosclerosis. HDL was shown to enhance fibrinolysis and inhibit platelet aggregation, suggesting the beneficial role of HDL in thrombotic processes [5•]. HDL inhibits in-vitro LDL oxidation induced by metal ions or endothelial cells resulting in reduced macrophage uptake and cholesterol accumulation [43]. Navab *et al.* [44] have reported that HDL completely inhibits the endothelial transmigration of monocytes induced by minimally-oxidized LDL. The antioxidant properties of HDL has been recently attributed to its association with enzymes (*eg*, paraoxonase and platelet-activating factor acetylhydrolase), which hydrolyze or inactivate peroxides and oxidized phospholipids [45]. Additionally, HDL inhibits proinflammatory cytokine-mediated expression of endothelial adhesion molecules that are associated with monocyte infiltration [46]. These studies clearly suggest the beneficial effects of HDL on pathobiologic vascular events.

Metabolic processes in apolipoprotein AI and high-density lipoprotein synthesis and catabolism

In healthy individuals, various factors are known to alter HDL-cholesterol levels [5]. For example, weight loss, exercise, ethanol, high-fat diet, and female gender are associated with increased HDL-cholesterol levels. Alternatively, obesity, physical inactivity, high-carbohydrate diet, cigarette smoking, and male gender are associated with lower

HDL-cholesterol levels. Although not clearly understood, regulation of HDL-cholesterol appears to have different regulatory controls than HDL–apo AI. Using genetic tools, recent studies have largely established the role of apo AI in the regulation of HDL levels and associated atherogenesis [47]. The overexpression of human apo AI in the atherosclerosis-susceptible high-cholesterol-fed mice protected them from developing atherosclerotic lesions [48]. Although apo AII also accounts for a significant protein mass in HDL (approximately, 30%), genetic manipulation of apo AII appears to have a deleterious effects on atherogenesis. Schultz *et al.* [49] compared the ability of overexpression of apo AI and apo AII in transgenic models to understand their contribution in atherogenesis. In these apo AI and apo AI/II transgenic mice, the levels of apo AI and HDL-cholesterol were similar. However, the apo AI/II transgenic mice had a 15-fold increase in atherosclerotic lesions over apo AI transgenic mice. Additionally, the apo AII transgenic mice developed atherosclerotic lesions on both normal chow diet and on high-cholesterol diet [50]. These studies suggested that apo AII may have antagonistic role in apo AI's antiatherosclerotic properties; however, more conclusive studies are needed.

Apolipoprotein AI synthetic processes

Although regulatory mechanisms resulting in altered apo AI or AII levels correlate with HDL-cholesterol levels, the cholesterol content of HDL particles is also independently regulated by at least four steps in the reverse cholesterol transport pathway. These include 1) the rate of cellular unesterified cholesterol efflux, the initial step in reverse cholesterol transport, 2) cholesterol esterification by lecithin cholesteryl acyl transferase, 3) transfer of cholesteryl esters from HDL to other lipoproteins by the cholesteryl ester transfer proteins, and 4) selective cholesterol removal by the SR–BI receptor. The following discussion briefly summarizes current information on the synthesis and catabolic steps in apo AI metabolism, which appears to be importantly linked to HDL physiology. It should be kept in mind that individual lipid and apolipoprotein components of HDL appear to have different metabolic controls about which much more research is needed.

The apo AI gene is expressed mainly in the liver and small intestine, and its promoter sequence is highly conserved in the specific region [51]. The current evidence indicates that there are three active sites within this conserved region that regulate hepatic apo AI gene transcription and expression [52,53]. Certain transcription factors of the steroid/thyroid hormone receptor superfamily members (*eg*, apo AI regulatory protein-1 and hepatocyte nuclear factor-4) can bind to specific apo AI promoter sites and modulate apo AI mRNA transcription activity and apo AI protein synthesis [51]. Thus, the alterations in apo AI gene promoter activity and modulations in regulatory transcription factors, by molecular or pharmacologic manipulations, may govern the apo AI synthesis and secretion. Additionally, the apo AI mRNA transcript degradative rate (*ie*, stability of mRNA) also play an important role in the final apo AI synthesis and secretory processes. Unlike apo B, post-translational apo AI or AII degradation is not an important regulatory mechanism for these major apoproteins of HDL.

Apolipoprotein AI catabolic processes

Alternatively, the plasma levels of apo AI and HDL are also regulated by the catabolic rates of apo AI/HDL. The liver is the primary organ for HDL and apo AI catabolism. Although various processes involved in the catabolism of HDL/apo AI are not clearly established, recent studies have characterized a scavenger receptor type B class I (SR–BI) in liver and steroidogenic tissue that mediate cholesterol ester uptake [54]. The SR–BI receptor was shown to bind HDL and to mediate the selective uptake or removal of HDL-cholesterol esters without the uptake/degradation of HDL–apo AI. It has been proposed that the SR–BI receptor acts as a "docking" site so that cholesterol esters can be removed after which the HDL particle (depleted of cholesterol esters) "undocks" and recirculates to pick up more cholesterol esters from peripheral tissues. In fact, mice with decreased expression of SR–BI by targeted gene mutation showed increased HDL cholesterol levels and decreased selective uptake of HDL-cholesterol esters in the liver [55,56]. Overexpression of SR–BI resulted in decreased HDL cholesterol, increased selective uptake of HDL cholesterol esters, and increased biliary cholesterol content, suggesting an enhancement in RCT pathway [57,58]. Furthermore, recent studies have addressed the biological significance of SR–BI receptor in atherogenesis [59•]. This study indicated that the transgenic mice with liver-specific overexpression of SR–BI crossed onto LDL-receptor-deficient background and showed significant reduction in atherosclerotic lesions when compared with LDL-receptor-deficient mice. The atherosclerotic protective effect appears to be primarily related to the lowering of VLDL and LDL cholesterol levels, whereas there was no relationship between the extent of atherosclerosis and HDL cholesterol levels. It was suggested that the LDL-lowering effects of SR–BI overexpression may be related to increased removal from the circulation either of LDL or its precursors, VLDL remnants [59].

In addition, there appears to be another receptor mechanism in liver associated with the removal of whole HDL particles. This putative novel "HDL–apo AI catabolism receptor" is not well defined currently, apart from the evidence suggesting that its ligand may be HDL–apo AI [60]. Thus, the current literature suggests that the HDL cholesterol and apo AI are catabolized by at least two different receptor pathways: one mediates selective cholesterol removal, whereas the other binds apo AI and mediates whole HDL particle elimination [5•]. It is possible that the up- or down-regulation of these catabolic pathways could influence HDL concentrations and the RCT pathway. Down-regulation of the putative "HDL–apo AI catabolic receptor" mediating whole HDL particle uptake (but not the SR–BI receptor) would result in increased apo AI concentrations and augmentation of reverse cholesterol transport. As SR–BI receptor appears to exert multiple lipoprotein

uptake (*eg,* HDL-cholesterol esters, LDL/oxidized LDL), the independent relevance of SR–BI in HDL-mediated cardioprotective events warrants further research.

The role of niacin in HDL and apo AI synthetic and catabolic processes

As discussed above, the plasma levels of HDL and apo AI are regulated by a balance between the synthetic and catabolic processes. Abnormalities in the regulation of either one or both of these two events in hyperlipidemic patients and the modulation of these processes by pharmacologic agents would govern HDL levels. Earlier kinetic studies have shown abnormalities in either synthetic or catabolic rates of HDL/apo AI in various metabolic diseases with low HDL levels, including primary HDL deficiency, hypertriglyceridemia, Tangier's disease, LCAT deficiency, diabetes, obesity, and renal disease [5]. Niacin has been commonly used for many years to regulate abnormalities in plasma lipid metabolism and in the treatment of atherosclerotic cardiovascular disease [5]. It is the most potent clinically used agent that increases circulating HDL-cholesterol and apo AI. Recent studies indicate that niacin increases LPA-I more than LPA-I and AII [5].

In early in vivo kinetic studies in humans, the mechanism of action of niacin to raise HDL and apo AI was mainly attributed to its ability to decrease the fractional catabolic rate of apo AI rather than alterations in synthetic rates in humans (Table 1) [61,62]. Using in vitro Hep G2 cell system, recently we have described a novel hepatocellular mechanism by which niacin increases apo AI and HDL (Table 1). In these studies, we have examined the effects of niacin on various steps involved in apo AI metabolism, including apo AI synthesis and secretion, mRNA expression, uptake of radiolabeled HDL-apo AI and HDL-cholesterol esters, and the properties of secreted particles to functionally efflux cellular cholesterol [63••]. The results indicated that niacin increased the accumulation of apo AI in Hep G2 cell culture media. Incorporation of radiolabeled-leucine and methionine (assessed by pulse–chase methods) into apo AI was unchanged by niacin, suggesting that niacin had no effect on apo AI synthetic processes. The ability of niacin to increase apo AI in the culture media without affecting apo AI synthesis led us to hypothesize that niacin may have a role in the removal or reuptake of HDL by hepatocytes. Using radiolabeled HDL-apo AI and HDL-cholesterol esters, we have shown that niacin specifically inhibited the uptake of HDL-apo AI but not HDL-cholesterol esters [63••]. Additionally, the conditioned media obtained from Hep G2 cells treated with niacin resulted in an increase in cholesterol efflux from the cultured fibroblasts (Table 1) [63••]. Thus, the results of these in vitro studies indicate that niacin selectively decreases the hepatic removal of HDL-apo AI, but not HDL-cholesterol esters, thereby increasing the capacity of retained apo AI to augment reverse cholesterol transport.

As shown in the proposed model (Fig. 1), the in vivo mechanisms of niacin to reduce plasma TG and VLDL levels are attributed to the ability of niacin to inhibit VLDL–TG synthetic rate (transport) and reduced fatty acid mobilization from adipocytes in patients with hyperlipidemia. Our in vitro studies in hepatocytes showing the direct inhibitory role of niacin in TG synthesis support the in-vivo kinetic observation of decreased VLDL–TG synthetic rate in niacin-treated patients [33]. Because niacin inhibited both fatty acid synthesis and esterification of fatty acid to form TG, it is conceivable that niacin may inhibit key committed enzyme systems involved in lipid synthesis (*eg,* fatty acid synthase, DGAT, and so forth). We have further proposed that the ability of niacin to increase post-translational apo-B degradation in hepatocytes would be the major mechanism of action of niacin to reduce VLDL and LDL levels observed clinically after niacin treatment. Based on our in vitro data in hepatocytes, we propose that the niacin-mediated inhibition of TG synthesis and availability may limit apo-B lipidation leading to the delayed translocation of apo B across the ER membrane, which in turn may increase intracellular apo-B degradation (Fig. 1). Additionally, niacin-mediated inhibition of TG synthesis and limited availability of TG may inhibit the generation of large TG-rich VLDL1 particles (which are precursors of small dense LDL particles), but instead may generate dense TG-poor VLDL2 particles. These VLDL2 particles by CETP-mediated processes generate TG-poor LDL particles, which are not preferred substrate for hepatic lipase. Thus, LDL particles remain more buoyant than the generation of small, dense LDL particles (Fig. 1). Alternatively, niacin-mediated inhibition of TG may generate decreased concentrations of VLDL1 particles. This may inhibit the formation of small, dense LDL particles (Fig. 1). This novel concept derived from studies in Hep G2 cells would support the clinical observations of niacin-mediated inhibition of VLDL–TG transport, decreased plasma VLDL and TG levels, and more buoyant LDL particles (rather than small, dense LDL particles with higher atherogenecity) in patients with hyperlipidemia.

Based on our in vitro studies in hepatocytes, we propose that niacin, by selectively inhibiting the hepatic removal of HDL-apo AI (but not HDL-cholesteryl ester), increases apo AI and HDL levels (Fig. 1). Because niacin appears to selectively increase LPA-I versus LPA-I+AII, the cholesterol content in HDL may be decreased because LPA-I has been shown to be a more efficient cholesterol ester donor (via probably SR–B1 receptor) than LPA-I+AII. Other unidentified mechanisms (*eg,* via CETP) may also exist and need to be assessed. Our studies also suggest that niacin inhibits the removal of HDL-apo AI at the level of putative HDL catabolism receptor or pathways, but not SR–BI-mediated events, which are selective to HDL-cholesterol esters (Fig. 1) [5•]. This proposed mechanism also explains the decreased fractional catabolic rate of apo AI previously observed in turnover studies in niacin-treated patients [61,62]. The increased HDL-apo AI particles initiate and promote cholesterol efflux, and thereby facilitate

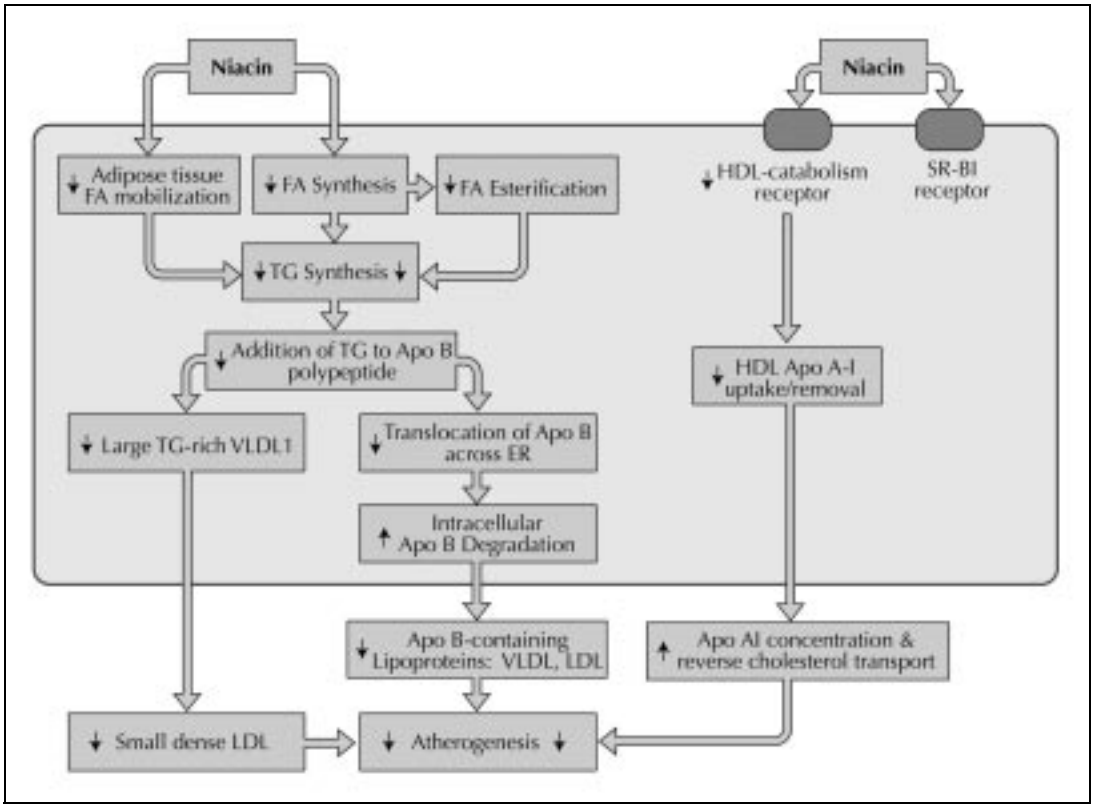

Figure 1. Proposed model of the mechanism of action of niacin on intracellular apo B and apo AI metabolic processes in hepatocytes. Niacin, by inhibiting fatty acid and TG synthesis, increases intracellular apo B degradation in hepatocytes. Niacin has also been shown to decrease fatty acid release from adipose tissue, and which may further reduce fatty acid availability for TG synthesis. Niacin-mediated inhibition of TG synthesis may decrease apo B lipidation associated with delayed translocation of apo B across ER membrane resulting in increased intracellular apo B degradation. Increased hepatocyte apo B degradation by niacin would decrease the number of VLDL (and their catabolic product, LDL) particles secreted, and explain the lower apo B and LDL concentrations observed clinically after niacin treatment. Additionally, niacin-mediated inhibition of TG synthesis and its availability may produce TG-poor VLDL2 particles (rather than large TG-rich VLDL1 particles), which may in turn produce more buoyant LDL particles rather than small dense LDL particles. Alternatively, niacin-mediated inhibition of TG may generate decreased concentrations of VLDL1 particles, and which in turn may inhibit the formation of small dense LDL particles. Evidence suggests that niacin inhibits the putative "HDL catabolism" pathway involving apo AI but not the SR-BI receptor that mediates selective HDL-cholesterol ester removal. Such mechanisms of decreased catabolic processes by niacin would increase apo AI and HDL thereby augmenting reverse cholesterol transport and other HDL-related vascular beneficial events. Thus, niacin, by these collaborative intracellular metabolic processes, favorably modulates LDL and HDL levels resulting in decreased atherosclerotic coronary disease. ER—endoplasmic reticulum; FA—fatty acid; TG—triglyceride

the removal of excess cholesterol from peripheral tissues through reverse cholesterol transport pathway. The removal of excess cholesterol from peripheral tissues, including arteries, may subsequently reduce the atherogenesis and cardiovascular disease. This is in addition to other beneficial effects described earlier (*eg,* antioxidant property).

Thus, our in vitro studies in hepatocytes along with earlier in vivo studies by others provide novel mechanisms by which niacin beneficially modulates VLDL, LDL, and HDL metabolic events, thereby reducing and preventing pathobiologic processes associated with atherosclerosis (Fig. 1). Further research is clearly needed to confirm these concepts in other in vitro primary hepatocytes and in vivo systems to increase our understanding of these complex areas of lipoprotein metabolism. Such mechanistic information could lead to identification of novel classes of drugs with similar mechanisms of action or targeting the specific cellular event in lipoprotein metabolic pathway. In the absence of new drug discoveries, more effective elevations of HDL and reductions in VLDL and LDL could be achieved by combining currently available drugs with different mechanisms of action. For example, a combination of gemfibrozil or estrogens that stimulate apo AI hepatic production could be combined with niacin, which decreases apo AI catabolism, to boost HDL levels above that of either drug alone. Likewise, the combination of HMG-CoA reductase inhibitors that primarily act by increasing LDL catabolism (via upregulating LDL receptors) could be combined with niacin to potentially yield profoundly beneficial decreases in the apo-B containing lipoproteins (VLDL, LDL, LP[a] and small, dense LDL) and potently increase HDL, HDL2, apo AI, and LPA-I. Ongoing clinical trials of these combinations

support these proposed drug combinations, yielding clinical approaches that are more potent in preventing atherosclerotic cardiovascular disease than combination therapy aimed at lowering mainly LDL.

Acknowledgment

The studies presented in this review were supported by Kos Pharmaceuticals, and the Long Beach Research Foundation.

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