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Niacin and cholesterol: role in cardiovascular disease (Review)

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Abstract

Niacin has been widely used as a pharmacologic agent to regulate abnormalities in plasma lipid and lipoprotein metabolism and in the treatment of atherosclerotic cardiovascular disease. Although the use of niacin in the treatment of dyslipidemia has been reported as early as 1955, only recent studies have yielded an understanding about the cellular and molecular mechanism of action of niacin on lipid and lipoprotein metabolism. In brief, the beneficial effect of niacin to reduce triglycerides and apolipoprotein-B containing lipoproteins (e.g., VLDL and LDL) are mainly through: a) decreasing fatty acid mobilization from adipose tissue triglyceride stores, and b) inhibiting hepatocyte diacylglycerol acyltransferase and triglyceride synthesis leading to increased intracellular apo B degradation and subsequent decreased secretion of VLDL and LDL particles. The mechanism of action of niacin to raise HDL is by decreasing the fractional catabolic rate of HDL-apo AI without affecting the synthetic rates. Additionally, niacin selectively increases the plasma levels of Lp-AI (HDL subfraction without apo AII), a cardioprotective subfraction of HDL in patients with low HDL. Using human hepatocytes (Hep G2 cells) as an in vitro model system, recent studies indicate that niacin selectively inhibits the uptake/removal of HDL-apo AI (but not HDL-cholesterol ester) by hepatocytes, thereby increasing the capacity of retained HDL-apo AI to augment cholesterol efflux through reverse cholesterol transport pathway. The studies discussed in this review provide evidence to extend the role of niacin as a lipid-lowering drug beyond its role as a vitamin. Published by Elsevier Inc. All rights reserved.

1. Background

Nicotinic acid (Niacin, Vitamin B3) is a water soluble vitamin. The major metabolic role of niacin is that it serves as a precursor for two essential coenzymes, Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Both NAD and NADP can be reduced to NADH and NADPH, respectively, and these coenzymes participate in oxidation-reduction reactions catalyzed by dehydrogenase and oxidoreductase enzymes. These NAD/NADP linked enzyme systems are involved in virtually every aspect of metabolic processes. Clinically, niacin deficiency causes pellagra characterized by dermatitis, diarrhea and dementia. Vitamin and the nutritional related role of niacin has long been known and reviewed extensively elsewhere [1,2]. In this article, we review the current understanding of the effect of niacin on cholesterol and lipoprotein metabolism to highlight the role of niacin beyond the vitamin to the cardiovascular disease. This article is divided into 4 sections, including: I) Role of cholesterol and lipid-carrying lipoproteins in coronary heart disease (CHD), II) Niacin as a lipid-regulating agent and its effect on atherosclerotic cardiovascular disease, III) Mechanism of action of niacin on triglyceride and apo B metabolism, and IV) Role of niacin in high density lipoprotein (HDL) metabolism.

2. Role of lipids and lipoproteins in CHD

2.1. Lipid profile and CHD

Disorders in lipid (e.g., cholesterol and triglycerides) and lipoprotein metabolism are major established independent risk factors in the development and progression of atherosclerotic CHD. Lipid-carrying proteins, termed as lipoproteins, are classified as 3 major classes, including very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). VLDL carries mainly triglycerides, and cholesterol is carried mainly in LDL, and to a lesser extent in HDL particles. HDLs transport cholesterol from peripheral tissues to the liver for excretion and

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recycling. Several epidemiological studies have clearly shown that plasma cholesterol, triglycerides, and LDL are positively correlated to the development of atherosclerotic CHD, whereas HDL is negatively correlated to CHD (reviewed in 3). Because of this differential correlation to CHD, LDL-cholesterol is generally termed as "bad cholesterol" and HDL-cholesterol as "good cholesterol". For example, a curvilinear relation between total cholesterol and CHD mortality was clearly demonstrated in a large number of patients [4]. The beneficial effect of lowering plasma cholesterol and specifically LDL-cholesterol to decrease or prevent CHD progression in hyperlipidemic patients has been clearly demonstrated in primary and secondary prevention trials [3,5,6]. In some epidemiological studies, such as Framingham and Prospective Cardiovascular Munster studies, plasma levels of triglycerides and triglyceride-containing VLDL particles are also shown to be positively related to the development of CHD (reviewed in 5). The data from the Monitored Atherosclerosis Regression Study showed that plasma triglycerides and the ratio of total cholesterol/HDL-cholesterol were correlated to the lesion progression in mild-to-moderate atherosclerotic lesions (reviewed in 5). Elevated plasma levels of lipoprotein(a) have also been associated with premature CHD [7].

Contrary to the positive correlation of LDL, HDL bears an inverse or negative relationship to atherosclerotic CHD. Numerous epidemiologic studies have shown that the decreased levels of HDL are correlated with progression of CHD [3,8]. Additional prospective clinical studies confirmed these observations and established that the amount of HDL-cholesterol as a fraction of total cholesterol was an important inverse determinant of CHD risk [8]. The recently reported Veterans Affairs HDL Intervention Trial (VA HIT) clearly showed for the first time that increasing HDL-cholesterol without altering LDL-cholesterol in patients treated with gemfibrozil resulted in significant reductions in the risk of major cardiovascular events [9,10]. These epidemiological and interventional trials clearly suggest that HDL serves as an anti-atherogenic lipoprotein, and highlight the beneficial effects of increasing HDL levels in the management of CHD.

2.2. Mechanistic role of lipoproteins in the pathogenesis of CHD

The classical view of atherosclerosis has changed considerably during the past 5-10 years. The original understanding of atherosclerosis processes include: a) the lipid-laden material builds up on the surface of passive artery wall, b) the lipid build up or deposit (plaque) grows and eventually closes off an affected artery, c) the obstructed arteries limit blood supply to the target tissues, and d) the subsequent loss of viability of the blood-starved tissue. These events result in several atherosclerotic cardiovascular disease complications including myocardial infarction, stroke, angina etc. Understanding the dynamic nature of vascular wall cells (versus previous notion as a passive carrier of blood) changed this original view considerably, and recent studies suggest that the changes in arterial wall cell interaction with blood components would lead to the initiation and progression of atherosclerosis. The current concepts suggest that the atherosclerotic vascular disease is characterized by: vascular endothelial activation and dysfunction, accumulation of fat-laden deposits within the arteries, monocyte-endothelial interaction and infiltration of monocytes, transformation of monocytes into lipid-laden foam cells, smooth muscle cell hypercellularity and intimal migration, dysregulated deposition and degradation of extracellular matrix proteins, and plaque rupture. Inflammatory mediators appear to play a significant role in the pathogenesis of atherosclerosis. These aspects of the evolution of atherosclerotic CHD are reviewed extensively elsewhere [11,12,13]. We discuss below how lipoproteins modulate these events in the development of atherosclerotic CHD.

The novel discovery of scavenger or acetyl-LDL receptors in macrophages provided an initial pathobiological role for atherogenic lipoproteins (e.g., modified LDL) in the transformation of monocyte-macrophages into lipid-laden foam cells of atherosclerotic lesions [14]. Subsequent discoveries made by Steinberg's group and other investigators have shown that the oxidative modification of LDL, either in the circulation or in the arterial wall, plays a critical role in facilitating the uptake of these oxidized-LDL by scavenger receptors on macrophages leading to the formation of lipid-laden foam cells within the affected arteries [14]. An updated "Response-to-Injury" hypothesis proposed by Ross and associates highlighted the interactive role of hyperlipidemia in endothelial-monocyte activation processes [15]. These investigators showed an increased adherence of circulating monocytes to the arterial endothelium in cholesterol-fed animals, suggesting that hypercholesterolemia or atherogenic lipoproteins may activate the endothelium to produce various new cytokines and growth factors (reviewed in 15,16). Subsequent studies using both in-vitro and in-vivo model systems indicated that oxidatively-modified LDL and its components can activate endothelial cell expression of various adhesion molecules, monocyte chemoattractants, and growth factors involved in endothelial migration and accumulation of monocytes (reviewed in 17). Furthermore, atherogenic lipoproteins (e.g., LDL and oxidized forms) are shown to down regulate endothelial nitric oxide synthase and nitric oxide (vasodilator) levels and increased endothelin (vasoconstrictor) concentrations, which are known characteristic features of endothelial dysfunction [18]. These studies 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 anti-atherogenic properties of HDL have been mainly attributed to the ability of apoprotein A-I (major protein of HDL) containing HDL particles to initiate cholesterol efflux and facilitate the removal of excess cholesterol from peripheral tissues (such as arteries) and its delivery to the liver for removal through reverse cholesterol transport pathway (reviewed in 8). Additionally, HDL enhances fibrinolysis and inhibits platelet aggregation, suggesting the beneficial role of HDL in thrombotic processes [8]. HDL also potently inhibits LDL oxidation induced by metal ions or endothelial cells resulting in reduced macrophage uptake and cholesterol deposition [19]. Recent studies indicate that HDL completely inhibits the endothelial transmigration of monocytes induced by minimally-oxidized LDL [20]. HDL inhibited pro-inflammatory cytokine-mediated expression of endothelial cell adhesion molecules, which are involved in monocyte-endothelial interaction and monocyte infiltration [21]. It has been demonstrated that the anti-oxidant properties of HDL are attributed to its association with certain enzyme (e.g., paraoxonase and platelet-activating factor acetylhydrolase), which hydrolyze or inactivate peroxides and biologically active oxidized phospholipids [22]. These studies suggest the beneficial effects of HDL on some of the atherosclerotic processes, and may even antagonize or inhibit deleterious effects of LDL particles on pathobiologic events of atherosclerosis. Thus, beneficial modulation of LDL and HDL particles by pharmacologic agents or dietary supplementation would be of considerable importance in retarding or reversing atherosclerosis and CHD.

3. Niacin as a lipid-regulating agent and its effect on atherosclerotic CHD

The use of niacin as a pharmacologic agent has been reported as early as 1955 by Altshul, Hoffer and Stephen [23], and currently it is a widely used agent in the treatment of dyslipidemia [24,25]. In pharmacologic doses (1-3 g/day), niacin reduces concentrations of total plasma cholesterol, apolipoprotein (apo) B, triglyceride, VLDL, LDL, and Lp(a), and increases HDL levels (reviewed in 25). Niacin is the most potent available lipid-regulating agent to increase HDL levels. Because of these diverse effects on lipid profile, niacin is considered as the broad-spectrum lipid-regulating agent. Several clinical trials (secondary prevention and angiographic studies) indicate that the treatment with niacin significantly reduces total mortality, coronary events, and retards the progression and induces regression of coronary atherosclerosis (reviewed in 25). Although the use of niacin in the past has been associated with adverse effects (e.g., flushing and hepatic toxicity), recent studies utilizing newer formulations of niacin have shown significant reduction in flushing with minimal to no hepatic toxicity with comparable effects on plasma lipid profile (reviewed in 25). These newer formulations of niacin have considerably renewed interest in the use of niacin as a broad-spectrum lipid-regulating agent and particularly for raising HDL.

4. Mechanism of action of niacin on triglyceride and apo B metabolism

There are mainly two mechanisms by which niacin influences plasma lipids and the secretion of apo B bearing lipoproteins including VLDL particles in the liver. VLDL is precursor to subsequent catabolic products including VLDL remnants, intermediate density lipoproteins (IDL), and LDL. Lp(a) is essentially LDL with a polypeptide linkage of apo B to apo(a). These include: 1) modulation of triglyceride lipolysis in adipose tissue, and 2) modulation of triglyceride synthesis resulting in increased intracellular apo B degradation.

4.1. Modulation of triglyceride lipolysis in adipose tissue

One mechanistic strategy to decrease elevated levels of lipids (e.g., triglycerides) in blood may be the inhibition of lipolysis in adipose tissue. Adipose cells are specialized for the synthesis and storage of triglycerides and for their mobilization to the liver as a fuel in the form of free fatty acids and glycerol. Triglycerides are highly concentrated store of metabolic energy in adipose tissue. As noted in Fig. 1, earlier studies indicated that niacin decreased the mobilization of fatty acids from adipose tissue by inhibiting the lipolysis of triglycerides [26]. Adipose tissue triglyceride lipolysis is generally controlled by c-AMP mediated activation of hormone sensitive lipase (reviewed in 1,2). Previous studies showed that niacin inhibited adenylate cyclase activity in adipocytes resulting in reduced concentrations of c-AMP [27]. Additionally, niacin was also shown to inhibit forskolin-induced c-AMP production in rat adipocytes [28]. Since c-AMP production can be regulated by G-protein receptor-dependent/independent mechanisms, niacin may use some of these mechanisms in inhibiting triglyceride lipolysis in adipose tissue. Although niacin has been shown to bind to certain G-protein coupled receptors in adipocytes and spleen (but not in other tissues, 29), the characterization of specific receptor for niacin or the participation of this receptor in niacin-mediated adipocyte triglyceride lipolysis is unclear.

4.2. Modulation of triglyceride synthesis and secretion of VLDL particles by liver

Liver is the primary organ for the production and secretion of apo and its associated lipids and ultimately VLDL and Lp(a) particles. However, the intestine also produces lipoproteins of similar size and lipid composition with lower molecular weight truncated apo B [48]. The secreted VLDL particles, by peripheral (adipose tissue, muscle) lipoprotein

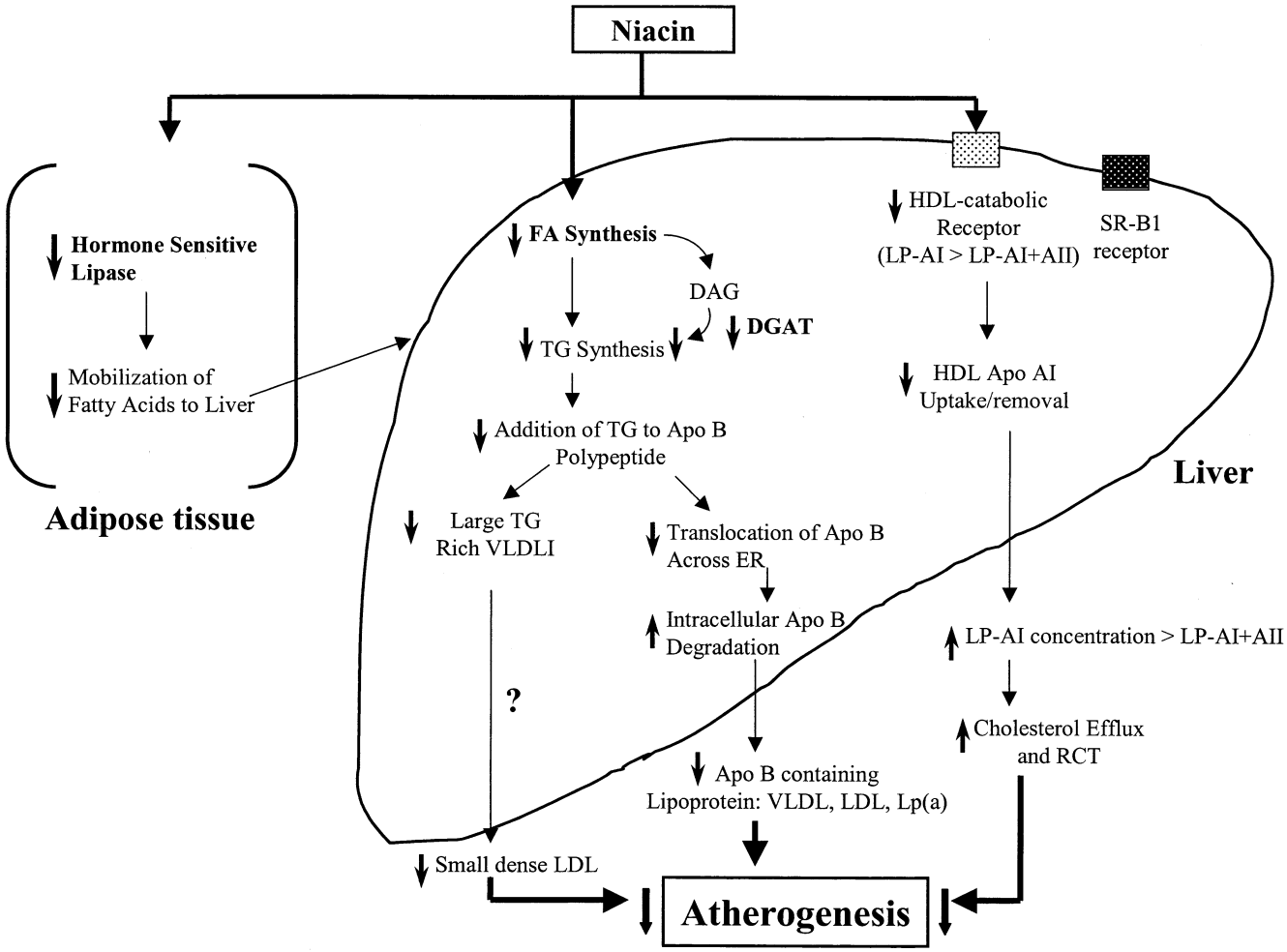

Fig. 1. Overview of current concepts on mechanism of action of niacin on lipid and lipoprotein metabolism. Niacin, through hormone-sensitive lipase-mediated events, inhibits fatty acid mobilization from adipose tissue. Inhibition of fatty acid (FA) release from adipose tissue results in decreased availability of fatty acids for triglyceride (TG) synthesis. In hepatocytes, niacin inhibits fatty acid synthesis and the esterification of diacylglycerol (DAG) to form TG mediated by direct non-competitive inhibition of diacylglycerol acyltransferase (DGAT) resulting in decreased TG synthesis. Niacin-mediated inhibition of TG synthesis may decrease the lipidation of apo B and translocation through endoplasmic reticular (ER) membrane leading to increased intracellular apo B degradation. Increased hepatocyte apo B degradation by niacin would decrease the number of VLDL and their catabolic product, LDL particles, which explains the lower apo B and LDL concentrations observed clinically after niacin treatment. Additionally, niacin-mediated inhibition of TG synthesis may produce decreased concentrations of large TG-rich VLDL1 particles, and which in turn may result in decreased formation of small dense LDL particles. In-vitro studies in hepatocytes suggest that niacin inhibits the putative "HDL catabolism receptor" involving removal of HDL-apo AI, but not the SR-BI receptor that mediates selective HDL-cholesterol ester removal. These mechanisms of decreased HDL-apo AI catabolism by niacin would increase HDL half-life and its concentration thereby augmenting cholesterol efflux and reverse cholesterol transport (RCT), and other HDL-related vascular beneficial effects. Increased residence time would also allow HDL size to increase (HDL2 > HDL3) from peripheral tissue cholesterol uptake. Taken together, niacin, through these collaborative intracellular metabolic processes, favorably modulates LDL and HDL levels resulting in decreased atherosclerotic coronary artery disease.

lipase-mediated triglyceride hydrolysis, are converted to intermediate density lipoprotein (IDL) and subsequently to LDL particles. The hepatic intracellular processing of apo B (the major protein of VLDL and LDL) plays a central role in regulating apo B lipoprotein secretion (reviewed in 30–32). The major regulatory processes in intracellular apo B processing and VLDL/LDL secretion include: localization of newly synthesized apo B as it translocates across the endoplasmic reticular (ER) membrane, the post-translational apo B degradation, and the mechanisms governing the synthesis and addition of core lipids to the nascent VLDL particles for secretion [31,32]. Current evidence indicates that a large amount of de novo synthesized apo B is not secreted, but rather is post-translationally degraded in hepatocytes. Additional studies have 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 (reviewed in 31,32). It has been suggested that the prolonged association of apo B with the ER membrane targets apo B for degradation, while rapid translocation of apo B across ER membrane facilitates apo B secretion as VLDL particles. These intracellular apo B processing and

its degradation 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. Of particular relevance to this article, the rate of lipid synthesis and the availability to lipidate apo B play a critical role in the translocation of apo B resulting in either secretion or intracellular degradation prior to secretion. Oleic acid (which increases triglyceride synthesis and secretion) has been shown to stimulate apo B secretion from hepatocytes by facilitating the translocation of newly synthesized apo B away from ER proteases, and thereby protecting newly synthesized apo B from intracellular degradation [33]. Further supporting evidence for the participation of triglyceride in apo B degradation was provided by demonstrating that the inhibition of fatty acid and triglyceride synthesis inhibited apo B secretion [34]. However, the role of cholesterol in apo B degradation is controversial [17,35]. Below, we discuss the effect of niacin on some of the above processes to understand the mode of action of niacin on VLDL and LDL secretion.

In earlier studies, Grundy and associates examined the influence of niacin on cholesterol and triglyceride metabolism using plasma turnover kinetic studies in humans [36]. In these studies, treatment of hyperlipidemic patients with niacin significantly decreased triglycerides in plasma by 52% and in VLDL by 36%. Multicompartmental analysis of plasma kinetic data following injection of ^{3}H-glycerol as a precursor indicated that niacin decreased synthetic rate (transport) of VLDL-triglycerides by 21%. Plasma kinetic data also suggested that the triglyceride reduction was mainly due to a decrease in triglyceride content of VLDL. In these studies, the reduction in plasma cholesterol was not associated with changes in fecal excretion of cholesterol or bile acids. It was noted that niacin induced a small significant increase in hepatic secretion of biliary cholesterol that may, at least in part, explain the loss of cholesterol from the body [36] and possibly represent enhanced reverse cholesterol transport. Since reduction of plasma cholesterol by niacin is mainly associated with LDL, reduced LDL particle production from VLDL may play a significant role in niacin-mediated reduction in plasma cholesterol. These in-vivo kinetic studies did not indicate the cellular and molecular targets of niacin's effect.

In order to gain insight into the hepatocellular mechanism of action of niacin on VLDL/LDL metabolism, we have recently reported experimental evidence addressing the effect of niacin on regulatory processes involved in intracellular apo B degradation and secretion in human hepatocyte cell line (Hep G2 cells), which has been used extensively as a model system for studying hepatocellular lipoprotein metabolism. Niacin increased apo B intracellular degradation and decreased subsequent secretion of apo B into the culture media of Hep G2 cells [37]. Niacin did not alter either the steady-state expression of apo B or the uptake of LDL by Hep G2 cells. We have also shown that niacin had no effect on MTP activity, suggesting normal triglyceride transfer activity in niacin-treated cells. Studies assessing the effects of niacin on protease-mediated apo B degradation were performed using ALLN (N-acetyl-leucyl-leucyl-norleucinal), a protease inhibitor. As expected and similar to earlier studies, treatment of Hep G2 cells with ALLN significantly decreased apo B degradation when compared to control cells. However, the apo B degradation in ALLN and ALLN plus niacin treated cells was not significantly different. These data suggest that the effect of niacin to induce apo B degradation is independent of ALLN-inhibitable protease-mediated pathways. Using oleic acid (which increases triglyceride synthesis, and previously shown to decrease intracellular apo B degradation), we have also examined whether alterations in the newly synthesized triglyceride and its availability would be involved in niacin-induced apo B degradation. The data indicated that niacin decreased inhibition of oleate-mediated apo B degradation, suggesting that niacin-induced apo B degradation may be dependent on the pathways involving the synthesis and association of triglyceride prior to apo B processing. To demonstrate the direct effect of niacin on triglyceride synthesis, we examined the incorporation of radiolabeled acetate, oleate, or glycerol into newly synthesized triglycerides in Hep G2 cells. The data indicated that niacin significantly inhibited triglyceride production at two synthetic sites: 1) fatty acid synthesis from acetate, and 2) esterification of fatty acids to form triglyceride [37]. Niacin had no effect on cholesterol synthesis in these cells. Additionally, in preliminary studies, we have shown that niacin directly in a non-competitive type inhibited microsomal diacylglycerol acyltrasferase (DGAT), a key rate-limiting enzyme in triglyceride synthesis [38]. These observations suggest that niacin by inhibiting DGAT decreases triglyceride synthesis and its availability in hepatocytes resulting in increased apo B degradation and subsequent decreased secretion of VLDL/LDL particles. It is attractive to suggest that although niacin is an old and established drug for lipid regulation, it may be representative of a potentially new class of agents that lower apo B atherogenic lipoproteins by DGAT inhibition. These experiments also indicate a major therapeutic target explaining its mechanism of action.

5. Role of niacin in HDL metabolism

As discussed earlier, niacin is the most effective pharmacologic agent to increase HDL levels. HDL are a complex class of lipoproteins with hydrophobic core of cholesterol esters and triglycerides and an outer hydrophilic layer of apolipoproteins, phospholipids and unesterified cholesterol. Apo AI and apo AII are the major proteins of HDL (accounting for approximately 70% and 20% of protein mass respectively). The liver and intestine are major organs for synthesis and secretion of HDL and its components. The plasma levels of HDL and its components are finely regulated by various synthetic and catabolic processes (reviewed

in 30). HDL particles circulating in the plasma consist of heterogeneous population, and are classified mainly by physical and chemical composition. HDL2 (density, 1.063-1.125 g/ml) and HDL3 (density, 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 apo AII (LP-AI) and particles containing both apo AI and apo AII (LP-AI+AII). It has been suggested that these HDL subfractions have certain different metabolic regulation and physiologic functions and also their association to atherogenesis (reviewed in 30). General consensus is that LP-AI particles are more potent in effluxing cellular cholesterol than LP AI+AII particles [39]. Furthermore, studies have shown that LP-AI particles are more efficient donors of cholesterol esters (CEs) than are LP-AI+AII particles [40]. Clinical studies have indicated that the increased levels of LP-AI particles are associated negatively with the degree of arteriographically defined coronary disease [41,42]. Although niacin has long been used specifically to raise HDL, only recently studies are beginning to address the cellular mechanisms of action of niacin on HDL metabolism (Reviewed in 30). Early plasma turnover studies in humans indicated that niacin primarily decreased the fractional catabolic rate of apo AI without altering apo AI synthetic rates [43,44]. Using human hepatocytes (Hep G2 cells), recently we have performed various studies to address the hepatocellular mechanism of action of niacin to modulate various steps involved in apo AI metabolism, including apo AI synthesis and secretion, uptake of HDL particles, and the properties of secreted particles to functionally efflux cellular cholesterol [45]. The data indicated that niacin increased the accumulation of apo AI in Hep G2 cell culture media. However, niacin did not affect the de novo synthesis of apo AI (as measured by the incorporation of radiolabeled leucine or methionine into newly synthesized apo AI) and the mRNA expression of apo AI [45], suggesting that niacin has no effect on apo AI synthetic processes. Therefore we hypothesized that niacin may influence the removal or reuptake of HDL by hepatocytes. Using radiolabeled HDL-apo AI and HDL-cholesterol, niacin selectively inhibited the uptake of HDL-apo AI but not HDL-cholesterol esters [45]. These data suggest that niacin may increase the capacity of retained apo AI to augment cholesterol efflux and reverse cholesterol transport pathway. Based on these data we suggest that niacin inhibits the removal of HDL-apo AI at the level of yet unidentified putative "HDL holoparticle catabolism receptor" or pathways, but not SR-B1-mediated events, which is selective to HDL-cholesterol esters [46]. It is conceivable that cubilin (which has been shown to bind to HDL-apo AI and mediate endocytosis in yolk-sac endoderm like cells, [47,48] may participate in niacin-mediated HDL-apo AI uptake, however the expression and the involvement of cubilin in hepatic uptake of HDL has not yet been established. The proposed cellular mechanism also explains the decreased fractional catabolic rate of apo AI previously observed in turnover studies in niacin-treated patients. Further turnover studies in humans are needed to simultaneously study kinetics of apo AI versus cholesterol esters after niacin treatment, which may shed light on potential receptor-mediated HDL catabolism.

To address the effect of niacin on HDL subfractions, we have recently examined the effect of extended-release niacin or gemfibrozil on LP-AI and LP-AI+AII (HDL particles containing apo AI + AII) levels in 139 patients with low HDL-cholesterol in a multicenter double-blind trial [49]. The data indicated that during the 19-week treatment period niacin dose-dependently and significantly increased LP-AI levels by 24% above baseline. Gemfibrozil had no significant effect on LP-AI. Treatment of patients with niacin also significantly increased LP-AI+AII particles by 9.5% above baseline and similar to the effect of gemfibrozil; however, the effect was less compared with LP-AI levels. These data suggest that niacin significantly increased LP-AI particles, a cardioprotective subfraction of HDL in patients with a low HDL state. As an extension of our previous study [45], we proposed that the increase in LP-AI particles in niacin-treated patients might be due to decreased hepatic removal/uptake of LP-AI particles. Indeed, data from in-vitro studies with Hep G-2 cells indicated that niacin significantly inhibited the uptake of radiolabeled LP-AI particles by Hep G2 cells [49]. However, niacin had no significant effect on the uptake of LP-AI+AII particles by Hep G2 cells. The uptake of HDL-cholesterol esters was approximately 75% greater from LP-AI versus LP-AI+AII particles, but niacin (or gemfibrozil) had no effect on LP-AI-cholesterol ester uptake by Hep G2 cells. These data suggest that niacin, by selectively inhibiting the hepatic removal/uptake of LP-AI particles, may lead to the increased retention of LP-AI particles in the circulation. The observation of greater cholesterol ester uptake from LP-AI particles compared with LP-AI+AII would highlight the beneficial properties of LP-AI particles as a better donor for cholesterol esters for reverse cholesterol transport pathway. Thus, by selectively decreasing hepatic LP-AI uptake/removal, niacin may have a greater effect not only on LP-AI mass but also in reverse cholesterol transport function. Although these in-vitro data provide novel approach for the mechanism of action of niacin to raise LP-AI HDL particles, additional direct plasma kinetic studies with these HDL subfractions in humans would be warranted to address the synthetic or catabolic aspects of these particles in control and niacin-treated patients.

6. Conclusions

A brief outline of the mechanisms of action of niacin on triglyceride, VLDL/LDL, and HDL metabolic processes is shown in Fig. 1. The ability of niacin to reduce triglyceride and VLDL/LDL secretion may involve modulation of spe-

cific events in adipocytes and hepatocytes. Firstly, niacin can decrease the mobilization of fatty acids from adipose tissue by inhibiting the hormone-sensitive lipase-mediated lipolysis of triglycerides. Secondly, niacin by directly inhibiting hepatic microsomal diacylglycerol acyltransferase (DGAT, a key enzyme in triglyceride synthesis) inhibits hepatic triglyceride synthesis, which limits apo B lipidation, resulting in delayed translocation of apo B across the endoplasmic reticular membrane. Increased intracellular apo B degradation and subsequent decreased VLDL/LDL and possibly Lp(a) secretion would result. Additionally, niacin-mediated inhibition of triglyceride synthesis and limited availability of triglyceride may decrease the generation of large triglyceride-rich VLDL1 particles, which are precursors of small dense LDL particles. The decreased levels of VLDL1 particles may inhibit the formation of atherogenic small, dense LDL particles (Fig. 1). This concept, developed mainly in Hep G2 cells provides mechanistic rationale and supports clinical observations about niacin-mediated inhibition of VLDL-triglyceride transport, decreased plasma apo B containing atherogenic lipoproteins i.e. VLDL, LDL and small dense LDL particles in patients with dyslipidemia [50].

Based on our in-vitro studies in Hep G2 cells, we propose that niacin by selectively inhibiting the hepatic removal/uptake of HDL-apo AI (but not HDL-cholesterol ester) retains increased plasma concentrations of HDL-apo AI (Fig. 1). The studies also suggest that niacin inhibits the removal of HDL-apo AI through the yet unidentified *putative HDL catabolic receptor,* but not through SR-B1-mediated pathways (Fig. 1). This proposed model also explains the decreased fractional catabolic rate of apo AI previously observed in plasma kinetic studies in niacin-treated patients [43,44].

The in-vitro mechanistic studies in hepatocytes and previous in-vivo studies provide novel mechanisms by which niacin beneficially affect VLDL, LDL, and HDL levels. The decreased levels of VLDL, LDL, and small dense LDL particles in niacin-treated patients through some of the mechanisms discussed above may decrease the progression of atherosclerosis (Fig. 1). Alternatively, increased HDL-apo AI particles (such as LP-AI) in niacin-treated patients may initiate and promote cholesterol efflux, and thereby facilitate the removal of excess cholesterol from peripheral tissues (such as arteries) through reverse cholesterol transport. The removal of excess cholesterol from arteries may eventually decrease the atherogenesis and cardiovascular disease (Fig. 1). Based on these concepts derived from in-vivo and in-vitro studies discussed in this article, we propose that the role of niacin can be extended beyond that of a vitamin to its role in atherosclerosis and cardiovascular disease prevention.

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References

[1] Henderson LM. Niacin. Annu Rev Nutr 1983;3:289–307.
[2] McCormick DB. Niacin in modern nutrition in health and disease. In: Shils ME, Young VR, editors; Philadelphia: Lea and Febiger, 1988. p. 370–5.
[3] Wong ND, Kashyap ML. Cholesterol and lipids. In: Wong ND, editor. Preventive cardiology. New York: McGraw-Hill Companies, Inc., 2000. p. 165–94.
[4] For the MRFIT, Stamler M, Wentworth D, Neeaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart desease continuous and graded? Findings in 356, 222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 1986;256:2823–8.
[5] Kwiterovich PO Jr. State-of-the-art update and review. Clinical trials of lipid-lowering agents. Am J Cardiol 1998;82:3U–17U.
[6] Zambon A, Hokanson JE. Lipoprotein classes and coronary disease regression. Curr Opin Lipidol 1998;9:329–36.
[7] Scanu AM. Lipoprotein(a) A Genetic Risk Factor for Premature Coronary Heart Disease. JAMA 1992;267:3326–9.
[8] Kashyap ML. Mechanistic studies of high density lipoproteins. Am J Cardiol 1998;82:42U–48U.
[9] Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high density lipoprotein cholesterol: Veteran Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. New Eng J Med 1999;341:410–8.
[10] Robins SJ, Collins D, Wittes JT, et al. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: A randomized controlled trial. JAMA 2001;285:1585–91.
[11] Ross R. The pathogenesis of atherosclerosis-an update. N Eng J Med 1986;314:488–500.
[12] Ross R. Atherosclerosis-An Inflammatory Disease. New Eng J Med 1999;340:115–26.
[13] Libby P. Heart Disease. In: Brauneald E, Zipes DP, Libby P, editors. A text book of cardiovascular medicine. 6th ed, W. B. Saunders, 2001.
[14] Goldstein JL, Ho YK, Basu SK, et al. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoproteins, producing massive cholesterol deposition. Proc Natl Acad Sci USA 1979;76:333–7.
[15] Steinberg D. low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997;272:20963–6.
[16] Ross R. The pathogenesis of atherosclerosis, a perspective for the 1990s. Nature 1993;362:801–9.
[17] Kamanna VS, Bassa BV, Kirschenbaum MA. Atherogenic lipoproteins and human disease: extending concepts beyond the heart to the kidney. Currn Opin Nephrol and Hypertension 1997;6:205–11.
[18] Liao JK. Endothelium and acute coronary syndromes. Clin Chem 1998;44:1799–808.
[19] Banka CL. High density lipoprotein and lipoprotein oxidation. Current Opin Lipidol 1996;7:139–42.
[20] Navab M, Imes SS, Hama SY, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. J Clin Invest 1991;88:2039–46.
[21] Cockerill GW, Rye KA, Gamble JR, et al. HDL inhibits cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 1995;15:1987–94.

[22] Navab M, Hama SY, Hough GP, et al. High density associated enzymes. Their role in vascular biology. Current Opin Lipidol 1998; 9:449–56.
[23] Altschul R, Hoffer A, Stephen JD. Influence of nicotinic acid on serum cholesterol in man. Arch Biochem Biophys 1955;54:558–9.
[24] Weiner M, Eys V. In Nicotinic acid, nutrient-cofactor-drug. New York: Dekker, 1983. p. 227–99.
[25] Tavintharan S, Kashyap ML. The benefits of niacin in atherosclerosis. Current Atherosclerosis Reports 2001;3:74–82.
[26] Carlson LA, Oro L, Ostman J. Effect of single dose of nicotinic acid on plasma lipids in patients with hyperlipoproteinemia. Acta Med Scand 1968;183:457–65.
[27] Aktories K, Schultz G, Jakobs KH. Regulation of adenylate cyclase activity in hamster adipocytes. Naunyn-Schmiedeberg's Arch Pharmacol 1980;312:167–73.
[28] Lacasa D, Agli B, Giudicelli Y. Increased sensitivity of fat cell adenylate cyclase to stimulatory agonists during fasting is not related to impaired inhibitory coupling system. FEBS Lett 1986;202:260–6.
[29] Lorenzen A, Stannek C, Lang H, et al. Characterization of a G protein-coupled receptor for nicotinic acid. Mol Pharmacol 2001;59: 349–57.
[30] Kamanna VS, Kashyap ML. Mechanism of action of niacin on lipoprotein metabolism. Current Atherosclerosis Reports 2000;2:36–46.
[31] Ginsberg HN. Synthesis and secretion of apolipoprotein B from cultured liver cells. Current Opin Lipidol 1995;6:275–80.
[32] Davis RA. Cell and molecular biology of the assembly and secretion of apolipoprotein B-containing lipoproteins by the liver. Biochim Biophys Acta 1999;1440:1–31.
[33] Dixon JL, Furukawa S, Ginsberg HN. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from Hep G2 cells by inhibiting early intracellular degradation of apolipoprotein B. J Biol Chem 1991;266:5080–6.
[34] Wu X, Sakata N, Lui E, et al. Evidence for a lack of regulation of the assembly and secretion of apolipoprotein B-containing lipoprotein from Hep G2 cells by cholesteryl ester. J Biol Chem 1994;269: 12375–82.
[35] Cianflone KM, Yasruel Z, Rodriguez MA, et al. Regulation of apo B secretion from Hep G2 cells. Evidence for a critical role for cholesteryl ester synthesis in the response to a fatty acid challenge. J Lipid Res 1990;31:2045–55.
[36] Grundy SM, Mok HYI, Zech L, Berman M. Influence of nicotinic acid aon metabolism of cholesterol and triglycerides in man. J Lipid Res 1981;22:24–36.
[37] Jin FY, Kamanna VS, Kashyap ML. Niacin accelerates intracellular apo B degradaqtion by inhibiting triacylglycerol synthesis in human hepatoblastoma (Hep G2) cells. Arterioscler Thromb Vasc Biol 1999; 19:1051–9.
[38] Ganji SH, Tavintharan S, Zhu D, et al. Niacin non-competitively inhibits hepatocyte diacylglycerol acyltransferase, a key enzyme for triglyceride synthesis. Arterioscler Thromb Vasc Biol 2002;22:878 (Abstract).
[39] Barbaras R, Puchois P, Fruchart J-C, Ailhaud G. Cholesterol efflux from cultured adipose cells is mediated by LP A-I particles but not by LP A-I: A-II particles. Biochem Biophys Res Commun 1987;142: 63–9.
[40] Rinninger F, Kaiser F, Windler E, et al. Selective uptake of cholesteryl esters from high- density lipoprotein derived LP A-I and LP A-I: A-II particles by hepatic cells in culture. Biochim Biophys Acta 1998;1393:277–91.
[41] Puchois P, Kandoussi A, Fievet P, et al. Apolipoprotein A-I containing lipoproteins in coronary artery disease. Atherosclerosis 1987;68: 35–40.
[42] Amouyel P, Isorez D, Bard JM, et al. Parental history of early myocardial infraction is associated with decreased levels of lipoparticle A-I in adolescents. Arterioscler Thromb 1993;13:1640–4.
[43] Blum CB, Levy JR, Eisenberg S, et al. High density lipoprotein metabolism in man. J Clin Invest 1977;60:795–807.
[44] Shepherd J, Packard CJ, Patsch JR, et al. Effect of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein A metabolism. J Clin Invest 1979;63:858–67.
[45] Jin FY, Kamanna VS, Kashyap ML. Niacin decreases removal of high density lipoprotein apolipoprotein A-I but not cholesterol ester by Hep G2 cells. Implications for reverse cholesterol transport. Arterioscler Thromb Vasc Biol 1997;17:2020–8.
[46] Acton S, Riggoti A, Landschutz KT, et al. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 1996; 271:518–20.
[47] Hammad SM, Steingrimur S, Twal WO, et al. Cubilin, the endocytic receptor for intrinsic factor-vitamin B12 complex, mediates high-density lipoprotein holoparticle endocytosis. Proc Natl Acad Sci USA 1999;96:10158–63.
[48] Kozyraki R, Fyfe J, Kristiansen M, et al. The intrinsic factor- vitamin B12, cubilin, is a high affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. Nature Med 1999;5:656–61.
[49] Sakai T, Kamanna VS, Kashyap ML. Niacin but not gemfibrozil, selectively increases LP-AI, a cardioprotective subfraction of HDL, in patients with low HDL cholesterol. Arterioscler Thromb Vasc Biol 2001;21:1783–9.
[50] Zambon A, Hokanson J, Brown BG, Brunzell JD. Evidence for a new pathophysiological mechanism for coronary artery disease regression, hepatic lipase-mediated changes in LDL density. Circulation 1999; 99:1959–64.