Abstract and Introduction

Abstract

Dyslipidemia is a heterogeneous metabolic condition; high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein represent families of lipoprotein particles that differ in size and composition and vary in atherogenicity. Lipoprotein subclasses containing apolipoprotein B promote atherosclerosis, of which the most atherogenic appear to be the small, dense LDL and large very-low-density lipoprotein subclasses, while the large HDL2 subclass, which transports esterified cholesterol from the periphery to the liver, is considered the more cardioprotective.

Niacin has long been known to improve concentrations of all major lipids and lipoproteins, but it also has consistently favorable effects on subclass distribution. A MEDLINE search was conducted for clinical studies reporting the effects of niacin on lipoprotein subclasses. The niacin-associated elevations in HDL cholesterol likely stem from differential drug effects on subclasses, producing favorable changes in levels of HDL2 and apolipoprotein A-I. Niacin has more moderate LDL cholesterol-lowering efficacy, but this change is associated with an increase in LDL particle size and a shift from small LDL to the less atherogenic, large LDL subclasses. In addition, it also tends to decrease concentrations of the larger very-low-density lipoprotein subclasses. Niacin confers diverse benefits with respect to both the quantity and quality of lipid and lipoprotein particles.

Introduction

Epidemiologic evidence has long suggested that elevated serum levels of low-density lipoprotein (LDL) cholesterol and triglycerides (TG), as well as low levels of high-density lipoprotein (HDL) cholesterol, increase the risk of coronary heart disease (CHD). Moreover, decades of randomized, controlled trials have shown that lipid-modifying therapies, notably the statins, significantly reduce the rates of primary and secondary cardiovascular events by approximately 25%-35% compared with placebo. Nonetheless, cardiovascular risk in treated patients remains significant.

Dyslipidemia is a heterogeneous metabolic condition that involves a complex, interacting array of lipids and lipoproteins with varying influence on CHD risk. LDL, HDL, and very-low-density lipoprotein (VLDL) represent families of lipoprotein particles that differ in size, composition, and density and also vary in atherogenicity. It is therefore not surprising that drug therapy aimed at total lipid levels, which actually encompass a range of lipoprotein subclasses collectively measured by typically undiscriminating assays, may often fail to maximize risk reduction. More successful would be an agent or combination of agents that has favorable effects on subclasses and total lipid levels.

The lipid-modifying effects of niacin at pharmacologic dosages have been recognized for more than a half-century. Niacin has been shown to improve concentrations of all lipid-related CHD risk factors; it is the most powerful agent currently available for raising HDL cholesterol and also lowers TG, LDL cholesterol, and lipoprotein(a). It has also been shown to favorably influence the distribution of HDL, LDL, and VLDL sub-classes. Moreover, niacin therapy, alone or combined with other agents, has been associated with reversal of angiographic coronary artery disease progression and reductions in cardiovascular morbidity and mortality.

The increasing recognition of the importance of the lipid and lipoprotein subclasses in the atherogenic process is leading...
to renewed interest in the multidimensional properties of niacin. Therefore, it is timely to review the effects of niacin on lipoprotein subclasses.

**Importance of Lipoprotein Subclasses**

**Proatherogenic Subclass Particles**

Lipoprotein subclasses containing apolipoprotein (apo) B, including VLDL, intermediate density lipoprotein (IDL), and LDL, promote atherosclerosis.[12] Particles of VLDL vary in size depending on their TG content. Increased serum TG levels promote the hepatic formation of large VLDL. Large VLDL formation is accelerated in patients with insulin resistance, such as occurs in obesity, metabolic syndrome, and type 2 diabetes. These particles have proatherogenic properties, particularly via the generation of other atherogenic lipoproteins such as IDL, a remnant lipoprotein and precursor of LDL.[5,12] When large VLDL concentrations are high, the action of cholesterol-ester transfer protein exchanges TG in the largest VLDL particles for cholesterol esters on large LDL. With large LDL partially depleted of cholesterol esters, hydrolysis of its acquired TG by hepatic lipase leads to the formation of small, dense LDL particles.[5]

Although small, dense LDL particles have reduced concentrations of free cholesterol, cholesterol ester, and phospholipids compared with larger, more buoyant LDL particles, they represent the most atherogenic LDL fraction.[13,14] The difference in composition appears to be associated with the increased susceptibility of small, dense LDL particles to lipid peroxidation, which promotes inflammation and foam cell formation.[15] Small, dense LDL particles have reduced affinity for hepatic LDL receptors, which prolong their presence in the circulation. Their small size may be responsible for the facility with which they cross the endothelium and penetrate the vascular wall.[12,16] Oxidized LDL also inhibits nitric oxide production and promotes thrombosis.[17] Persons with a predominance of small, dense particles are classified as having LDL phenotype pattern B, compared with pattern A characterized by large LDL particle predominance. Family studies suggest that pattern B is inherited as a dominant single-gene trait with a population frequency of 25%.[18] In addition, chylomicrons are another form of TG-rich lipoprotein that are formed in the intestine from dietary fat. Chylomicron remnants probably also have some atherogenic potential.[1]

**Antiatherogenic Subclass Particles**

HDL particles transport cholesterol from peripheral cells to the liver, but the different HDL subclasses have varying involvement in this process of reverse cholesterol transport and disparate effects on atherogenesis. Cholesterol-poor small HDL (HDL₃) originates in the liver and intestines.[5,19] The HDL₃ core expands with the accumulation and subsequent esterification of free cholesterol by lecithin-cholesterol acyltransferase. The result is a larger, more buoyant, mature HDL₂ particle that transports esterified cholesterol to the liver. The HDL₂ subfraction also has antioxidant properties, inhibits thrombotic factors, and is considered the more cardioprotective.[19,20]

**Lipoprotein Subclasses and CHD Risk**

**LDL Subclass Studies**

Multiple studies have shown a strong relationship between small LDL particles and the development or progression of CHD. In a population-based study, subjects with smaller LDL particle size (<0.256 nm) had 2.2 times the risk of developing CHD compared with those with larger particle size, independent of all traditional lipid risk factors.[21] Moreover, the degree of risk attributable to LDL cholesterol, TG, and HDL cholesterol was significantly influenced by LDL size. Small LDL particle size was associated with increased risk even in patients with average LDL cholesterol levels. The Stanford Coronary Risk Intervention Project (SCRIP) found that baseline levels of small, dense LDL (LDL-IVb=0.220-0.233 nm) were the best predictor of coronary stenosis progression over 4 years.[22] The annual rate of progression was six times greater among CHD patients with LDL-IVb levels in the fourth quartile compared with the first quartile. Similar results were seen in the Pravastatin Limitation of Atherosclerosis in the Coronary arteries trial (PLAC-I), where small LDL was associated with a five-fold greater risk of angiographic progression.[23]

Support for a role for small, dense LDL as a potential target for therapy in CHD patients has come from a post-hoc analysis of the Familial Atherosclerosis Treatment Study.[24] Combination therapy with niacin-colestipol or...
lovastatin-colestipol increased LDL buoyancy, and this change was the most powerful predictor of coronary stenosis regression, accounting for 37% of the variance. Reductions in apo B accounted for only 5% of the variance while changes in other lipid and non-lipid risk factors had little effect. Similarly, in the Diabetes Atherosclerosis Intervention Study, fenofibrate treatment increased LDL size and decreased apo B-containing lipoproteins, and these changes were associated with decreased coronary stenosis progression. This suggests that both quality and quantity of LDL particles play a role in the increased CHD risk in diabetes.

The potential for risk stratification based on lipoprotein subclasses has been explored in other populations at increased CHD risk. In a study of postmenopausal women, elevations in small LDL, large VLDL, and number of LDL particles were each significant and independent correlates of extensive coronary artery calcification. Small LDL particle predominance is a hallmark of the atherogenic dyslipidemia associated with the metabolic syndrome. It has also been advanced as part of the definition of familial combined hyperlipidemia. Despite the accumulating evidence, other studies in both healthy and CHD populations have not found small LDL particles to be independently associated with CHD risk after controlling for other risk factors, leading to some uncertainty about its predictive and clinical value.

HDL and VLDL Subclass Studies

Multiple studies have shown a strong relationship between HDL particle size and CHD risk. In SCRIP, coronary stenosis progression was significantly related to baseline level of HDL$_{3a}$ ($p=0.02$) and inversely related to HDL$_2$ ($p=0.02$). Similar results were seen in PLAC-I. In a study from the Milwaukee Cardiovascular Data Registry, men with CHD and high levels of small HDL particles or large VLDL particles were three to four times more likely to have angiographic evidence of extensive disease than men with low levels. Moreover, men with both abnormalities were 15 times more likely to have severe disease. Support for a role for small HDL particle size as a potential target for therapy in patients with CHD has come from a post-hoc analysis of the HDL Atherosclerosis Treatment Study. Combination therapy with simvastatin-niacin decreased small HDL particles and increased large apo A-I containing HDL particles. Patients with elevated large HDL (third tertile) showed no progression, while those in the first tertile showed a 2.1% increase in stenosis. However, in a subanalysis of the Monitored Atherosclerosis Regression Study, coronary stenosis progression was most strongly correlated with low levels of HDL$_3$ and elevations of small VLDL, suggesting a protective role for these subclasses.

Mechanism of Action of Niacin on Lipoprotein Subclasses

Niacin produces large, rapid decreases in TG levels by inhibiting release of fatty acids from adipose tissue as well as hepatic synthesis of fatty acids and TG (Figure 1). Reduced TG synthesis is postulated to enhance hepatic degradation of apo B, the major lipoprotein component of VLDL, thereby reducing VLDL production and hence levels of IDL and LDL. The reduction in TG availability also results in production of smaller, TG-poor VLDL particles, with subsequent inhibition of small, dense LDL production. Niacin may elevate HDL cholesterol levels primarily by suppressing the hepatic removal of apo A-I, which increases levels of apo A-I as well as large apo A-I containing HDL particles. Niacin also appears not to inhibit hepatic removal of cholesterol esters, and so preserves the ability of retained apo A-I to augment reverse cholesterol transport. In clinical studies, niacin has been shown to raise HDL cholesterol levels 15%-35% and to lower TG levels 20%-50%, lipoprotein(a) 24%-38%, and LDL cholesterol 5%-25%.
Efficacy of Niacin on Lipoprotein Subclasses

HDL Subclasses Studies

Niacin therapy has differential effects on HDL, producing favorable changes in subclasses and apo A-I. In 41 patients with dyslipidemia who received crystalline niacin at 4000 mg/d for 6 weeks, mean serum levels of HDL cholesterol increased by 135% as assessed using ultracentrifugation techniques ($p < 0.001$).[35] A similar evaluation was performed in 23 patients with dyslipidemia using gradient gel electrophoresis. After 6 weeks of niacin 4000 mg/d,[7] the 45% increase in total HDL cholesterol level was predominantly attributable to a 126% rise ($p < 0.001$) in HDL$_2$ cholesterol. The contribution of HDL$_{2b}$ and HDL$_{2a}$ to total HDL mass increased by 144% ($p < 0.001$) and 17% ($p < 0.01$), respectively. These were paralleled by a 34% decrease in the HDL$_{3b}$ ($p < 0.001$) and 26% decrease ($p < 0.01$) in the HDL$_{3c}$ subparticle proportions.

Our group performed a post-hoc evaluation of baseline and 12-week plasma samples from 60 patients with dyslipidemia who had been randomized to receive once-daily extended-release niacin (niacin ER) at either 1000 mg or 2000 mg, or to placebo.[8] Using a proton nuclear magnetic resonance assay, in the 1000 mg/d niacin group, concentrations of HDL H5 and H4, the two largest of five measured HDL subparticles and the two that make up the more cardioprotective HDL$_{2ab}$ subfraction, were increased by 70% and 39%, respectively ($p < 0.001$ vs. placebo for both). In the 2000 mg/d group, increases were more pronounced at 89% and 75%, respectively ($p < 0.001$ vs. placebo for both). No significant changes were observed in levels of HDL H3, H2, or H1, which collectively correspond to the HDL$_{3abc}$ subfraction (Figure 2).
The Effects of Niacin on Lipoprotein Subclass Distribution

Figure 2. Effects of niacin on high-density lipoprotein subclasses. ER=extended-release. Reprinted with permission from *Am J Cardiol*. 2003;91:1432-1436.[8]

Studies have shown that different niacin formulations can have variable effects on HDL subparticle distribution. In a randomized comparison of crystalline niacin with a sustained-release niacin preparation, 71 patients with dyslipidemia were treated with 3000 mg/d for 6 months.[36] Mean HDL₂ cholesterol levels increased by 36% on crystalline niacin but decreased by 5% on sustained-release niacin (p<0.05). In contrast, a study comparing crystalline niacin with niacin ER in 223 patients with dyslipidemia showed equivalent efficacy.[37] Mean HDL₂ and HDL₃ cholesterol levels increased by 37% and 17%, respectively, on niacin ER compared with 33% and 16%, respectively, on crystalline niacin (Figure 3). In addition, apo A-I levels increased by 8% with niacin ER and 6% with crystalline niacin. A similar increase in apo A-I levels (7%), as well as a decrease in apo A-II (-21%), was reported in an early study of crystalline niacin involving five healthy adults.[38]

Figure 3. Effects of niacin on high-density lipoprotein (HDL) subclasses. ER=extended-release; IR=immediate-release or crystalline; *p<0.05 vs. niacin ER and niacin IR 1500 mg/d. Adapted with permission from *Metabolism*. 1998;47:1097-1104.[37]

**LDL Subclasses Studies**

Niacin shifts the distribution of LDL subclass from smaller toward larger particles and seems to have a more pronounced effect in patients with pattern B dyslipidemia than in those expressing the pattern A phenotype. One study evaluated 26 patients with CHD and dyslipidemia for changes in gradient gel electrophoresis-measured LDL subclass distribution 3-6 months after starting on crystalline niacin 3000 mg/d.[6] Compared with baseline, mean peak LDL particle diameter increased by 0.74 nm (p<0.0001) overall, and by 0.94 nm among the 17 pattern B patients compared with 0.36 nm among
the nine patients with the pattern A phenotype ($p=0.022$). In addition, mean HDL cholesterol level increased by 13.4 mg/dL ($p<0.01$) among pattern B patients but by only 0.7 mg/dL ($p=NS$) in pattern A patients.

In our post-hoc evaluation of 60 patients randomized to niacin ER or placebo, levels of the smaller LDL subparticles L1 and L2 tended to decrease while the larger L3 subparticles tended to increase at both niacin dosages, although these changes were not significant vs. placebo.\[8\] On the other hand, niacin decreased the number of LDL particles by 15% at 1000 mg/d and by 23% at 2000 mg/d ($p=0.002$ for both). Both dosages were also associated with significant increases in LDL particle size ($p=0.027$).

Niacin's effects on LDL subparticle size and distribution have also been evaluated in diabetic populations. In the open-label study of 42 diabetics, 6-8 weeks of crystalline niacin, mean dose 2765 mg/d, was associated with an 0.11 nm increase in mean peak LDL particle diameter ($p=0.0001$).\[39\] Mean small, dense LDL mass decreased by 44%, from 27 mg/dL to 15 mg/dL ($p<0.0001$). Similarly, in the evaluation of 23 persons with diabetes using niacin ER, mean peak LDL particle diameter increased a significant 0.9 nm and mean small, dense LDL mass decreased by 43% ($p<0.0001$ for both).\[40\] In two studies comparing niacin preparations with statins in patients with diabetes, niacin had a greater effect on LDL particle size, statins had a greater effect on particle number, and the combination conferred complementary benefits that corrected the entire atherogenic profile.\[41,42\]

VLDL Subclasses Studies

Large VLDL subparticles are frequently found in the presence of other lipoprotein disorders, so their independence as a risk factor is not established. However, studies that show a favorable treatment effect on VLDL-associated TG are suggestive, given the status of elevated TG as an emerging risk factor in the Adult Treatment Panel III guidelines.\[1\] In our study of 60 patients with dyslipidemia randomized to niacin ER or placebo, total VLDL-associated TG decreased 24% and 37% at 1000 mg/d and 2000 mg/d, respectively.\[8\] Of the VLDL subparticles V1 through V6 (numbered smallest to largest), the V2, V4, and V5 subfractions accounted for the overwhelming proportion of total VLDL. The lower-dose niacin regimen was associated with 25% and 34% decreases in the V4 and V5 subfractions, respectively, compared with baseline values. The corresponding decreases with higher-dose niacin reached 49% and 57%, respectively. In a diabetic study, levels of V2 through V6 were reduced by 25%-34% on crystalline niacin 3000 mg/d.\[41\]

Niacin therapy, therefore, has diverse beneficial effects on all lipoprotein subfractions that are consistent with its proven ability to lower the risk of angioGraphic and clinical CHD. Moreover, the combination of niacin and a statin may be among the best available treatment options for many patients with complex forms of dyslipidemia. Although the cutaneous side effects may discourage some patients, newer formulations may help reduce their impact.\[37\] Niacin can be used safely in the majority of patients, including those with type 2 diabetes, with appropriate medical care.\[43\]

Conclusion

The increasing recognition of the importance of the lipid and lipoprotein subclasses in the atherogenic process is leading to renewed interest in the properties of niacin. Niacin effectively modifies all major lipids and lipoproteins with respect to both their quantity and quality. It is the most effective agent currently available for raising low levels of HDL cholesterol, and this change is predominantly attributable to an increase in the larger cardioprotective HDL2 subclass and in the level of HDL apo A-I. Niacin has more moderate LDL cholesterol-lowering efficacy, but this change is associated with an increase in LDL particle size and a shift to the less atherogenic, larger LDL subclass. Furthermore, niacin's effects appear to be greater in individuals with LDL pattern B, characterized by a predominance of small, dense LDL particles. Niacin, alone or in combination, represents a valuable option for clinicians in the management of dyslipidemia.

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