EFFECTS OF HYPOLIPIDEMIC AND HYPOGLYCAEMIC DRUGS ON Atherogenic Low-Density Lipoproteins Particles in Type-2 Diabetes

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SUMMARY

Type-2 diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced high-density lipoproteins (HDL)-cholesterol, a predominance of small, dense low-density lipoproteins (LDL), and elevated triglycerides. These abnormalities occur even in prediabetes before blood sugars rise sufficiently to make the diagnosis of diabetes and this transition phase incurs important cardiovascular risk. This is the rationale for paying attention to dyslipidaemia through the use of the hypolipidaemic rather than hypoglycaemic drugs and once diabetes occurs it must be through both avenues. Yet, beyond the “quantity” of LDL, several lipid-lowering agents, and particularly statins, are only in part beneficial on the “quality” of LDL, so that their net effect on small, dense LDL is moderate; therefore, fibrates should be preferred and their modulation of LDL size in diabetic patients has been associated with a reduced risk for cardiovascular events. Among hypoglycaemic agents, insulin and metformin have shown a limited role on small, dense LDL, while pioglitazone is more beneficial. It remains to be tested by future studies the efficacy of incretin-based therapies on LDL subclasses, considering that preliminary studies have reported significant improvements by these agents on triglycerides and HDL-cholesterol plasma concentrations.

Key words: Diabetes, small, dense LDL, LDL size, therapy, prevention

Introduction

Type-2 diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced high-density lipoproteins (HDL)-cholesterol, a predominance of small, dense low-density lipoproteins (LDL), and elevated triglycerides, while LDL or total cholesterol are generally not increased.

There is evidence that each of the dyslipidemic features associated with type-2 diabetes led to an increased risk of cardiovascular disease, the leading cause of death in these subjects. Many studies have demonstrated an association between LDL size or density and cardiovascular diseases and other reports have further indicated that levels of small dense LDL are predictive of coronary events and that this is independent of other coronary disease risk factors. Although lowering LDL cholesterol is important in reducing cardiovascular disease morbidity and mortality, there are a number of other factors...
contributing to the disease process that can be favourably affected by drug therapy\(^{16}\). In fact, increasing evidence suggest that the “quality” rather than only the “quantity” of LDL exerts a great influence on the cardiovascular risk and it has been shown that at least part of the cardiovascular benefits observed in prevention trials can be attributed to pharmacological effects on atherogenic small, dense LDL\(^{19}\). LDL are not homogeneous particles but comprise multiple distinct subclasses that differ in size, density, physicochemical composition, metabolic behaviour and atherogenicity, with at least four major subspecies\(^{5}\).

**Atherogenicity of small, dense LDL**

Several reasons have been suggested to explain the enhanced atherogenicity of small dense LDL. Smaller, more dense LDL are taken up more easily by arterial tissue than larger LDL\(^{10}\), suggesting greater transendothelial transport. In addition, small, dense LDL have decreased receptor-mediated uptake and increased proteoglycan binding\(^7\). Sialic acid, due to its exposure at the LDL surface, plays a determinant role in the in vitro association of LDL with the polyamionic proteoglycans\(^{18}\) and it has been shown that sialic acid content of LDL particles of subjects with the predominance of small, dense LDL is reduced. Further, oxidative susceptibility increases and antioxidant concentrations decreases with decreasing LDL size\(^7\).

Altered properties of the surface lipid layer associated with reduced content of free cholesterol\(^{20}\) and increased content of polyunsaturated fatty acids\(^{11}\) might also contribute to enhanced oxidative susceptibility of small, dense LDL. Compositional changes in LDL particles may account for much of the increased atherogenicity found in diabetes. We have shown that the ratio of esterified to free cholesterol was increased in the LDL particle from diabetic subjects\(^{12}\). The implication of this finding was that the oxidizability of the LDL would be increased and we further reported that oxidized LDL was increased in type-2 diabetes\(^{19}\).

Another mechanism whereby the LDL particle may become more atherogenic is through the increased glycation of the particle, with the glycation being directly related to the degree of hyperglycemia, and glycation appears to make the LDL particle more sensitive to oxidation\(^{14,15}\).

To date, the association of the quality of LDL with cardiovascular diseases has been tested in over fifty studies, including cross-sectional and prospective epidemiologic as well as clinical intervention trials\(^{10}\); these studies overall suggest that small, dense exerts a direct influence on cardiovascular risk. For such atherogenic properties their predominance have been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III\(^{15}\) and it has been shown that even small increases in the concentration of small, dense LDL particles may substantially contribute to the determination of total cardiovascular risk\(^{18}\).

**The clinical significance of the therapeutical modulation of small, dense LDL**

Several studies have interestingly investigated if the therapeutic modification of LDL size may be significantly associated with reduced cardiovascular risk. Such investigations used arteriographic changes as outcome variables and have reported that benefit was concentrated in patients with a predominance of small, dense LDL who received treatment that tended to lower small, dense LDL. These studies included the “Stanford Coronary Risk Intervention Project” (SCRIP), the “Familial Atherosclerosis Treatment Study” (FATS) and the “Pravastatin Limitation of Atherosclerosis in the Coronary Arteries” (PLAC-I) trial\(^{17-19}\). Lovastatin was administered in the SCRIP (with bile acid-binding resins, niacin or fibrates) and in the FATS (with colestipol, versus niacin and colestipol), pravastatin was used in the PLAC-I.

The therapeutical modulation of LDL size was significantly associated with reduced cardiovascular risk at univariate analysis. In addition, at multivariate analyses with adjustments for confounding factors, changes in LDL size by drug therapy were the best correlates of changes in coronary stenosis in FATS\(^{19}\). In PLAC-I, using a logistic regression models that adjusted for lipid levels and other confounding factors, elevated levels of small LDL were associated with a nine-fold increased risk of CAD progression in the placebo group\(^{17}\). All these data suggest that the therapeutic modification of LDL size may be significantly associated with reduced cardiovascular risk, even after multivariate adjustment for confounding factors.

In addition, although not directly demonstrated by measuring LDL size, the modulation of small, dense LDL with fibrates probably contributed to the reduction of cardiovascular risk in two clinical tri-
als, the “Helsinki Heart Study” and the “Veterans Affairs High-density Lipoprotein Cholesterol Intervention Trials Study Group” (VA-HIT)(20-22).

Yet, although fibrates are more powerful than statins in improving LDL quality, existing evidence suggests that statins are more powerful agents in reducing cardiovascular morbidity and mortality. Fenofibrate seems to be very effective in lowering small dense LDL, but the FIELD study(29) showed no significant reduction in primary end point in type-2 diabetics (randomised to receive fenofibrate or placebo). However, due to the inclusion criteria, in this study were excluded most of the subjects with diabetic dyslipidemia that would have been benefit more by the fibrate therapy. It should be noted that several studies have failed to demonstrate cardiovascular outcome improvement with better control of diabetes from the blood sugar point of view perhaps because they have not managed the blood sugar sufficiently well.

Since the increased free fatty acids that hang on LDL in diabetes are responsible, at least in part, together with the increased esterified cholesterol in the LDL particle, for the atherogenicity of diabetic LDL particles(2), only a meticulous control of blood sugar may normalise the disturbance in free fatty acid metabolism.

The clinical relevance of small, dense LDL in type-2 diabetes

Hypertriglyceridemia, low HDL cholesterol and an increased fraction of small dense LDL particles characterise diabetic dyslipidemia(24-26), while LDL or total cholesterol are generally not increased in diabetics, except for a slight increase of LDL cholesterol in women(27). In addition, small dense LDL is associated with the cluster of risk factors that characterise the insulin resistance syndrome(28). Interestingly, subjects with predominance of small dense LDL have a greater than two fold increased risk for developing type-2 diabetes, independent from age, sex, glucose tolerance and body mass index. An increase of LDL size was associated with a 16% decrease in the risk of developing diabetes(29).

It has also been shown that patients with the insulin resistance syndrome tend to have a predominance of small, dense LDL(30) and this has been confirmed for diabetes, in both men and women(25,31). In addition, using a euglycemic clamp technique to categorise individuals as insulin-sensitive, insulin-resistant, or type-2 diabetics, more severe states of insulin resistance were associated with smaller LDL size. We further investigated the clinical significance of LDL size and LDL subclasses in diabetes type 2 patients(32); in our study, diabetic patients with manifest coronary heart disease (CHD) had decreased LDL particle sizes and more small, dense LDL as compared to diabetic patients without established CHD. Multivariate analysis revealed that LDL size was the strongest marker of CHD as compared to the other established cardiovascular risk factors, including plasma lipids and lipoproteins.

Carotid intima-media thickness (IMT) is considered a reliable surrogate marker of early atherosclerosis and it has been shown to correlate significantly with the presence of CHD and to predict coronary events(33-36). In addition, significant relationships of carotid IMT with other lipid parameters as LDL-cholesterol(37) and apoB(38) have been demonstrated. In our study described above(32), LDL size was significantly associated with carotid IMT in type-2 diabetes and LDL size was the second strongest predictor of IMT, after smoking, when compared to all other cardiovascular risk factors, as well as the strongest predictor of IMT among all lipid parameters. Therefore, LDL size seems to represent a marker of clinical apparent (CHD) and non-apparent (carotid IMT) atherosclerosis in type-2 diabetes.

The effects of hypolipidemic agents on small, dense LDL in type-2 diabetes

Hypolipidemic treatments are able to favourably modulate LDL size and subclasses in high risk patients. Regarding subjects with type-2 diabetes this seems particularly true for fibrates and less for statins(39) (see Table 1). Analysis of published studies revealed that atorvastatin represents the most effective agent among statins(38-40), while fenofibrate, bezafibrate and gemfibrozil are all very beneficial in modifying LDL size and subclasses towards less atherogenic particles(41-57).

Nicotinic acid has been found also effective but the extended-release form should be preferred for the reduced intolerance(58,59), while fish oils showed a very limited role(60-63). Data on the use of ezetimibe, a cholesterol absorption inhibitor, need to be confirmed by future studies(64).

Interestingly, it has been investigated if the therapeutic modification of LDL size by fibrates (fenofibrate) may be significantly associated with reduced cardiovascular risk in an intervention trial, using arteriographic changes as outcome variables.
Table 1: Therapeutic modulation of LDL size and subclasses by different hypolipidemic agents in subjects with type-2 diabetes (as modified from 39).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Drug</th>
<th>Benefit</th>
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<tr>
<td>Winkler [40]</td>
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<td>Fluvastatin</td>
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</tr>
<tr>
<td>Kazama et al. [41]</td>
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<td>Pravastatin</td>
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<td>Geiss [42]</td>
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<td>Farnier et al. [64]</td>
<td>2005</td>
<td>Ezetimibe</td>
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EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.
In the “Diabetes Atherosclerosis Intervention Study” the therapeutical modulation of LDL size was significantly associated with reduced cardiovascular risk at univariate analysis; in addition, using multivariate analyses with adjustments for confounding factors changes in LDL size were strong predictors for cardiovascular events. These data suggest that in patients with type-2 diabetes the therapeutic modulation of LDL size and subclasses by fibrates is effective and significantly associated with a reduced risk for cardiovascular events.

The effects of hypoglycaemic agents on small, dense LDL in type-2 diabetes

Few studies have assessed if classic hypoglycaemic agents, including insulin and metformin, are beneficial on LDL size and subclasses and overall their net effect seems to be moderate. Insulin has been shown to increase LDL size and reduce small, dense LDL in diabetic patients already receiving high doses of sulfonylureas; however, these changes were associated with reductions in levels of triglycerides and triglyceride-rich lipoproteins. In another study metformin increased LDL size, even though it was not significant; yet, the numbers were very small and troglitazone started at low doses. Acarbose may decrease the amount of small, dense LDL particles.

Further, several studies have evaluated the impact of thiazolidinediones (pioglitazone and rosiglitazone) on small, dense LDL in patients with type-2 diabetes (see Table 2) and available data suggest that pioglitazone significantly reduce these particles, while rosiglitazone not. However, it cannot be excluded that other factors influenced this opposite effect, including the laboratory methods used, the trials’ design, the extent of diabetes control or severity, the nutritional habit or the diabetes medications used concurrently.

Glitazones, and particularly pioglitazone, have specific effects on triglyceride and fatty acid metabolism, as compared to all the other hypoglycaemic agents, lowering plasma triglycerides and improving the quality of LDL through suppression of fatty acids and reduction in triglyceride-rich lipoproteins, particularly in the post prandial phase. In this view, the recent study performed by the GLAI investigators represents an important trial evaluating the effects of the two thiazolidinediones in a parallel-group design on plasma lipids and lipoproteins (including small, dense LDL) over the whole density range using nuclear magnetic resonance. This head-to-head study reported a more pronounced decrease in small, dense LDL particles following pioglitazone compared to rosiglitazone. We recently extended such observations in a prospective, randomized, crossover trial, in order to make a direct comparison between these two compounds and we found that pioglitazone resulted in a more prominent shift of LDL subfractions towards larger particles than rosiglitazone. Importantly, these effects were documented in the presence of a similar effect on glycaemic control and insulin sensitivity.

It remains to be tested the effects on LDL size and subclasses of the new antidiabetic-drugs modulating the incretin system, such as the glucagon-like peptide-1 (GLP-1) receptor agonists and the dipeptidyl peptidase-4 (DPP4)-inhibitors. These agents have shown a different impact on plasma lipids: exenatide seems to be able to lower total-cholesterol, LDL-cholesterol and triglyceride levels with a significant improvement in HDL-cholesterol, while the use of the DPP-4 inhibitors sitagliptin and vildagliptin led to some improvements in triglycerides and HDL-cholesterol (reviewed in). Perhaps the better glucose lowering effect of GLP1 agonists as compared to the DPP4 inhibitors and the weight reduction as compared to no weight change with DPP4 use accounts for a better effect on atherogenic dyslipidaemia with GLP1 agonists.

Conclusions

All the evidence to date demonstrates that improvement in hyperglycaemia affects the small vessel outcome. Yet, hypoglycaemia is a dangerous condition too. Meticulous control of blood sugar will control free fatty acid abnormalities, triglycerides and post-prandial lipoprotein abnormalities. These abnormalities occur even in prediabetes before blood sugars rise sufficiently to make the diagnosis of diabetes and this transition phase incurs important cardiovascular risk.

This is the rationale for paying attention to pre-diabetic dyslipidaemia through the use of the hypolipaemic rather than only hypoglycaemic drugs, and once diabetes occurs it must be through both avenues.

Beyond LDL-cholesterol levels, recent evidence suggests that the presence of atherogenic lipoproteins is strongly associated with cardiovascular risk. Therefore, assessment and management of atherogenic dyslipidaemia, and particularly of small,
dense LDL, may significantly contribute to reduce cardiovascular risk, especially in higher risk subjects, such as those with type-2 diabetes. Yet, statins and other hypolipidemic agents are able only in part to modulate LDL subclasses towards less atherogenic particles; therefore, fibrates should be preferred and their modulation of LDL size in diabetic patients has been associated with a reduced risk for cardiovascular events.

Among hypoglycaemic agents, insulin and metformin have shown a limited role on small, dense LDL, while pioglitazone is more beneficial. It remains to be tested by future studies the efficacy of incretin-based therapies on LDL size and subclasses, since preliminary studies have reported significant improvements by these agents on triglycerides and HDL-cholesterol concentrations.

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