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Clinical applications of advanced lipoprotein testing in diabetes mellitus

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Abstract

Traditional lipid profiles often fail to fully explain the elevated cardiovascular risk of individuals with diabetes mellitus. Advanced lipoprotein testing offers a novel means to evaluate dyslipidemia and refine risk estimation. Numerous observational studies have demonstrated a characteristic pattern of elevated levels of small, dense LDL particles, out of proportion to traditional lipid levels, in patients with both diabetes mellitus and the metabolic syndrome. Commonly used glucose and lipid-lowering agents have varied effects in patients with diabetes on both LDL and HDL subfractions. The exact role of advanced lipoprotein testing in patients with diabetes mellitus and the metabolic syndrome remains unclear but may offer improved assessment of cardiovascular risk compared with traditional lipid measurements.

Keywords

diabetes; lipoprotein; metabolic syndrome; particles

The prevalence of diabetes mellitus continues to rise, with the WHO estimating that the number of patients with diabetes worldwide will increase from 171 million in 2000 to 360 million in 2030. In addition, the demographic expected to grow most significantly are those patients with diabetes aged 65 years and over [1]. As the number of individuals with diabetes mellitus continues to grow and their life expectancy increases, healthcare providers will be faced with the resultant increase in the incidence of the microvascular and macrovascular sequelae of diabetes. Though chronic hyperglycemia contributes to dysfunction in various organ systems, cardiovascular disease (CVD) remains the largest contributor to morbidity and mortality among those with diabetes [2] and remains the leading cause of premature death in this high-risk population [3,4], being responsible for approximately 65% of total deaths [5]. Both the American Diabetes Association [6] and American Heart Association [7] have designated diabetic patients as high risk for CVD, even in the absence of prior coronary artery disease. Furthermore, an analysis of data from the Framingham Heart Study demonstrated that the proportion of heart disease attributable to diabetes mellitus has increased over time [8].

The mechanism by which diabetes confers increased cardiovascular risk remains unclear. However, similar to patients without diabetes, a large body of evidence suggests a central role for lipids contributing to CVD in those with diabetes, a pattern termed diabetic dyslipidemia. In addition, there is a proinflammatory milieu at the level of the vascular

endothelium due to the accumulation of advanced glycation end products [9] that stimulates accelerated atherosclerosis. Considerable work has demonstrated that the diabetic lipid profile possesses an intrinsic atherogenicity that is not present in those without diabetes.

As diagnostic modalities have advanced, the ability to characterize lipid abnormalities that confer increased cardiovascular risk has also improved. Most clinicians are familiar with traditional cholesterol measurements, namely total cholesterol, LDL-C, HDL-C and triglycerides (TGs). However, analysis of the heterogeneous particles that comprise each of these cholesterol categories offers the possibility of discovering novel diagnostic and therapeutic modalities in lipid management. A variety of methodologies exist for the analysis of lipid subfractions. Density gradient ultracentrifugation is the oldest of the lipid subfraction technologies [10], which functions by exploiting the size and density differences that characterize various lipid fractions. Gradient gel electrophoresis (GGE) was described by Krauss and Burke in 1982 as a means to isolate LDL lipid subfractions by charge and size and has been refined extensively in subsequent years [11]. Nuclear magnetic resonance (NMR) spectroscopy, the most recent of the subfraction technologies, provides a simultaneous quantification of the size and concentration of lipoprotein particles (Table 1 & Figure 1) [12].

Although these various lipid subfraction methodologies differ in technique, no one technique has taken precedence over the others, and even within the various techniques, there is still disagreement about the boundaries that precisely define each lipid subfraction [13]. This lack of standardization raises the possibility of ambiguity within the literature utilizing subfraction technology. A review by Chung *et al.* of nine head to head analyses of these methodologies revealed significant heterogeneity in the results obtained from each of these techniques [14]. Concordance between subfraction data from technologies varied considerably in the study, and others have suggested that a consensus reference standard should be established to facilitate harmonization of this emerging field [15].

Lipid synthesis initiates at the liver and from intestinal absorption with production of VLDL and chylomicrons, respectively. Each contains apolipoproteins as well, with VLDL containing apolipoprotein B-100 and chylomicrons possessing apolipoprotein B-48. Both particles are also enriched with abundant TGs and cholesterol. As TGs are removed from VLDL particles through the action of lipases, the VLDL composition is transformed to an intermediate-density lipoprotein (IDL). Further lipolysis of IDL produces LDL particles. On the surface of all the atherogenic lipoproteins, including VLDL, IDL, and LDL, resides apolipoprotein B (apoB), in contrast to HDL, which contains apolipoprotein A. Measurement of circulating levels of apoB thus provides an accurate assessment of the number of these atherogenic lipid particles as each of those particles possesses one molecule of apoB. Alternatively, non-HDL-C (nHDL-C) provides similar assessment of the total atherogenic lipid pool and is recommended by the National Cholesterol Education Program as a secondary lipid goal after achieving LDL-C goals, particularly in those patients with hypertriglyceridemia [16]. nHDL-C has been demonstrated to be a stronger predictor for CVD risk than traditional LDL-C [17], and elevated levels were still associated with an increased risk of coronary heart disease with LDL levels below 100 mg/dl [18]. However, many individuals who reach their respective nHDL-C targets nevertheless still possess an atherogenic lipid profile with residual risk [19]. Recent studies demonstrated that apoB may be superior to both nHDL-C and LDL-C as a predictor of CVD [20].

Standard lipid panels only provide evaluation of the total amount of cholesterol in each major circulating lipid fraction but no information about the individual particles that carry that particular type of cholesterol. Two individuals can possess the same total LDL-C, but disparate LDL particle (LDL-P) compositions (Figure 2).

Austin *et al.* utilized GGE to dichotomize these LDL phenotypes into Pattern A characterized by larger, buoyant LDL particles, and Pattern B, characterized by a smaller, dense LDL pattern [21]. Analysis of lipid subfractions by GGE in the Quebec Cardiovascular Study found that a preponderance of small, dense LDL was associated with a greater than threefold increase in the risk of ischemic heart disease [22].

It has also been demonstrated that small, dense LDL particles preferentially undergo endocytosis by macrophages and also are more susceptible to oxidation relative to larger, more buoyant particles. Both processes stimulate foam cell production in coronary atheromas and are possible mechanisms of the increased atherogenicity associated with pattern B. Though less well studied, HDL has similarly been deconstructed into various subfractions but with inconsistent findings. Some have found that the smaller HDL subfractions are associated with increased risk of coronary artery disease [23,24], while more recent analyses have noted that those small subfractions may protect against CVD by acting as antioxidants [25].

The atherogenic lipid profile in insulin resistant states

Diabetic dyslipidemia is classically characterized by an increase in VLDL-C, decreased production of HDL and a preponderance of small, dense LDL particles [26]. The relationship between insulin resistance and the Pattern B phenotype of LDL has been demonstrated in several observational studies across various diabetic populations (Table 2). Siegel *et al.* analyzed the nature of lipoprotein abnormalities using GGE in patients from the third examination of Framingham Offspring Study conducted from 1984 to 1987 [27]. A total of 174 men and women with diabetes mellitus were compared with 3757 control subjects. The prevalence of the Pattern B phenotype was significantly higher in both diabetic men (49 vs 33%; $p < 0.05$) and women (40 vs 11%; $p < 0.001$) compared with subjects without diabetes mellitus. Similar findings have been observed in smaller case-control studies [28,29]. Interestingly, a gender interaction was observed in a case-control study by Haffner *et al.* using ultracentrifugation [30]. They observed significantly lower LDL particle size in both men and women with diabetes ($p = 0.007$). However, the difference remained only in women when corrections were made for both TGs and HDL-C. A consistent and important observation in these observational studies was that glycemic control, whether measured as fasting blood glucose or hemoglobin A1c, was not significantly different in patients with Pattern A or B LDL phenotypes.

Several predictors of LDL phenotype were assessed by Friedlander *et al.* from the second examination (1994–1996) of the Jerusalem Diabetes Prevalence Study [31]. In almost 400 diabetic men and women, a variety of parameters including elevated TGs ($p < 0.001$), low HDL-C ($p < 0.001$), elevated fasting insulin ($p = 0.005$), and 2 h postload insulin ($p = 0.02$) were associated with smaller LDL particle size as measured by GGE. Selby *et al.* similarly analyzed predictors of the Pattern B phenotype in twin comparisons from the Kaiser Permanente Women Twins Study conducted from 1989 to 1990 [32]. Hypertriglyceridemia ($p < 0.0001$), low HDL-C ($p < 0.0001$), elevated waist:hip ratio ($p < 0.0001$), and increased fasting insulin ($p < 0.0001$) were associated with the Pattern B phenotype assessed by GGE. Of note, a sub-group analysis of 25 pairs of nondiabetic, monozygotic twins discordant for LDL phenotype revealed elevated BMI and waist:hip ratio in the twin with a small, dense LDL pattern.

Isolated lipid abnormalities are rare in those with diabetes mellitus. More commonly observed is the ‘lipid triad’ of hypertriglyceridemia, small LDL particles, and low HDL, which together confer an increased risk of CVD [33]. An excess of TG-rich lipoproteins and concomitant elevated serum TGs results in a pattern shift to smaller LDL particles along

with lower overall HDL particle concentrations. Each of these characteristic lipid abnormalities are related to the underlying insulin resistance, characteristic of both the metabolic syndrome (MetSyn) and overt diabetes mellitus. Drexel *et al.* found that a small LDL particle diameter ($p = 0.002$), low HDL particle concentration ($p = 0.001$), low apoA-I ($p = 0.003$), and elevated TGs ($p = 0.007$) predicted vascular events in a population of 491 Caucasian patients with angiography-proven coronary artery disease on statin therapy [34]. This relationship persisted in the 116 patients with diabetes mellitus in the analysis. Similarly Berneis *et al.*, in a study of 38 Swiss individuals with diabetes mellitus demonstrated that LDL particle size was the strongest predictor of the presence of coronary artery disease ($p = 0.002$) and carotid intima-media thickness ($p = 0.03$) relative to other traditional lipid parameters such as total cholesterol, LDL-C, HDL-C, apoB, apoA-I and apoA-III [35].

As mentioned before, the prognostic importance of HDL subfractions in patients with diabetes has been less well studied. Syvanne *et al.* studied HDL subfractions using GGE in 50 consecutive patients with diabetes undergoing elective coronary catheterization [36]. Patients with both diabetes mellitus and significant coronary artery disease found at cardiac catheterization had smaller HDL particle size relative to patients with neither coronary artery disease nor diabetes or just one of those conditions ($p = 0.04$). Furthermore, an analysis by Williams *et al.* of 116 male and 78 female patients found that the proportion of HDL particles in the HDL 3b subfractions correlated positively ($p < 0.01$) with small LDL, TGs, and apolipoprotein B particle concentrations [37]. HDL particles are also intimately involved in mediating oxidation throughout the body. A recent study by Stefanovic *et al.* of 114 individuals with diabetes mellitus demonstrated an increased prevalence of the smaller HD3 particles relative to controls (28.3 vs 4.4%; $p < 0.001$) [38]. Those with the predominant HDL3 phenotype, the majority of which possessed diabetes mellitus, had elevated levels of superoxide anions and increased activity of superoxide dismutase. These findings suggest an adaptation to a molecular environment in diabetes mellitus of oxidative stress and provide a physiologic explanation for varying particle sizes in individuals with diabetes mellitus.

The lipoprotein subfraction abnormalities of diabetic dyslipidemia are observed even in prediabetic subjects. The Insulin Resistance Atherosclerosis Study analyzed lipid subfractions by NMR in a cohort of 830 individuals without diabetes at baseline [39]. There was no significant difference in baseline LDL-C noted in those that developed diabetes mellitus ($n = 130$) over the mean follow-up of 5.2 years relative to those who remained free from diabetes. However, they did manifest a discordant phenotype with significantly smaller baseline LDL and HDL particle size ($p = 0.0004$ and $p < 0.0001$, respectively) and significantly increased baseline levels of total LDL-P, small LDL-P, small HDL-P, and VLDL size. These lipid abnormalities suggest that healthy prediabetic individuals possess an atherogenic lipid profile prior to development of overt diabetes mellitus. Furthermore, several studies using a variety of lipid subfraction technologies have demonstrated that a preponderance of small LDL [40], small HDL, and VLDL particles [41] can predict the future development of diabetes mellitus.

Not only do atherogenic lipoprotein subfraction patterns predict prevalence and incidence of diabetes, but atherogenic LDL particles also directly promote vascular dysfunction in diabetic patients. Lu *et al.* isolated various electronegative LDL subfractions by means of fast protein liquid chromatography in six diabetic patients [42]. The L5 electronegative subfraction, initially observed in hyperlipidemic nondiabetic patients [43], was also found in those with diabetes mellitus. This subfraction dysregulates FGF-2 function and potentiates subsequent endothelial cell apoptosis. Another study using density gradient ultracentrifugation determined that LDL isolated from diabetic subjects had a significant

effect in decreasing the ability of animal endothelium to appropriately relax in the setting of cholinergic administration [44]. Finally, decreasing LDL particle size has been correlated with impaired endothelial flow mediated vasodilation in patients with and without diabetes mellitus ($r = 0.48$, $p < 0.05$; $r = 0.39$, $p < 0.0005$, respectively) [45]. Together, these studies suggest that the LDLs of diabetic patients may intrinsically confer cardiovascular risk by disrupting autoregulation at the microvascular level.

The MetSyn, characterized by dyslipidemia and central adiposity, is on an earlier spectrum of insulin resistance ending with diabetes. Analysis of 641 patients (376 men, 265 women) with MetSyn and 2352 controls from the Framingham Heart Study found that both men and women with MetSyn had a comparatively increased number of small LDL particles ($p < 0.0001$) and significantly less large LDL particles ($p < 0.0001$) as determined by NMR spectroscopy [46]. The number of small LDL particles increased in a step-wise fashion with increasing components of the MetSyn. A smaller study of 105 individuals with the MetSyn versus 70 age and sex-matched controls using GGE found a significant increase in cholesterol concentration within dense LDL, a decrease in mean particle size, and a decrease in LDL peak particle size (all parameters with $p < 0.05$) in those with MetSyn [47]. Rizzo *et al.* demonstrated in an analysis of 124 Italian patients with MetSyn that an elevated concentration of small, dense LDL was associated ($p = 0.004$) with an increased incidence of cardiovascular and cerebrovascular events at 2 years of follow-up [48]. The increasing epidemic of MetSyn [49] and obesity will likely lead to an increasing number of diabetic patients in the future and subsequent CVD, with a significant number characterized by abnormal LDL subfraction patterns out of proportion to their LDL-C levels.

Otvos *et al.* recently reported data from the Multi-Ethnic Study of Atherosclerosis (MESA) study to compare LDL-P derived from NMR spectroscopy and LDL-C as a predictor of incident CVD in a community based cohort of 6814 ethnically diverse individuals [50]. Specifically, the study focused on prognostic utility of LDL-P in individuals with discordant levels of LDL-P and LDL-C (defined as a difference of ≥ 12 percentile units). While both LDL-P (hazard ratio (HR) per standard deviation (SD) 1.32, 95% CI: 1.19–1.47; $p < 0.0001$) and LDL-C (HR per SD 1.20, 95% CI 1.08–1.34, $p = 0.0009$) independently predicted incident CVD in the overall population, only LDL-P retained its prognostic significance (HR per SD 1.45, 95% CI: 1.19–1.78, $p = 0.0003$) when the LDL-P and LDL-C values were discordant. Though LDL-C and LDL-P were highly correlated ($r = 0.75$), discordance was markedly higher in those with diabetes mellitus and MetSyn. In particular, discordance characterized by LDL-P greater than LDL-C suggests the presence of numerous, cholesterol-poor LDL particles representative of the Pattern B phenotype. Compared to individuals with concordant LDL-P and LDL-C, the 1407 subjects with discordant LDL-P $>$ LDL-C in the MESA study had markedly increased prevalence of diabetes mellitus (19%) and MetSyn (54%; $p < 0.0001$ for both comparisons). This large population-based study thus suggests an increasing role for measuring LDL subfractions in those with insulin resistance as a prognostic index of coronary artery disease beyond the traditional lipid profile (Tables 3 & 4).

Therapies & lipid subfraction changes

Therapies used in patients with diabetes targeted at both hyperglycemia and diabetic dyslipidemia have potential in altering the lipoprotein subfraction composition to a more favorable phenotype (Table 5 & Figure 3). Generally, therapies which reduce TG concentration have been demonstrated to have the most potent effect in reducing the proportion of small, dense LDL. However, the vast majority of literature to date in this area has comprised relatively small cohorts. Additionally, the correlation of changes in these

lipid subfractions to actual cardio vascular outcome measures in patients with diabetes has yet to be clarified.

Primary glucose-lowering therapies

Lifestyle changes

In one lifestyle study assessing the effects of weight reduction and dietary modifications in 34 German patients with Type 2 diabetes mellitus [51], an exercise regimen of 2200 kcal/week and a concomitant diet of 1000 kcal/day caused a significant reduction in small LDL particles (1.42–0.93 mg/dl; $p = 0.001$) relative to baseline measured by density gradient ultracentrifugation. Wagner *et al.* analyzed the effects of aggressive control of hyperglycemia by means of therapeutic lifestyle changes in combination with initiation of oral hypoglycemic or insulin therapy in a prospective trial of 33 patients with poorly controlled diabetes mellitus [52]. A significant reduction in A1c (10.5–7.0%; $p < 0.0005$) was achieved and, while apoB and LDL-C decreased, no change in LDL particle size was observed by means of GGE among all patients. However, when considering just those 14 of 33 patients with the Pattern B phenotype at baseline, a significant increase in LDL particle size was noted (from 25.1 to 25.6 nm; $p < 0.005$). However, another prospective analysis that assessed the impact of improved glycemic control in 70 individuals with poorly controlled diabetes mellitus found that those patients that transitioned from the Pattern B to Pattern A phenotype detected by ultracentrifugation also had larger decreases in hemoglobin A1c (4.9 vs 3.1%; $p < 0.05$) [53]. Finally, Rivellese *et al.* studied insulin versus sulfonylurea administration in nine patients with diabetes mellitus and determined that insulin use was significantly associated with a decrease in small and increase in large LDL particle subfractions as measured by ultracentrifugation ($p < 0.03$) [54].

Metformin

The different classes of oral hypoglycemic agents also have characteristic effects on the lipid profile of patients with diabetes. Metformin use was evaluated in a prospective study of 28 Japanese patients who were on prior sulfonylurea monotherapy [55]. LDL relative mobility (LDL-Rm), a measurement of LDL movement with polyacrylamide gel disc electrophoresis that is inversely related to particle size, was utilized to quantify LDL particle size. Use of metformin resulted in an increase in LDL particle size (LDL:Rm ratio 0.3521:0.3339; $p < 0.05$) compared with baseline, a particle size change that was independent of the improvement in glycemic control.

Thiazolidinediones

The effects of thiazolidinediones (TZD) on diabetic dyslipidemia have been more extensively studied. In a small study of eight diabetic patients assessing the effects of troglitazone [56], LDL particle size determined by NMR spectroscopy increased from 20.5 to 21.3 nm ($p < 0.05$) over 8 weeks of therapy, a change that was driven mainly by an increase in large LDL particle concentration (from 53 to 86 mg/dl; $p < 0.05$). The LDL particle size subsequently decreased to baseline after troglitazone therapy had been discontinued for 4 weeks. Lawrence and associates also compared both pioglitazone and metformin administration versus sulfonylurea utilization [57]. A total of 60 diabetic patients were randomized to gliclazide, pioglitazone, or metformin therapy. Glycemic control was equivalent across the three treatment groups, yet disparate effects on lipid subfractions were observed. Significant decreases in small LDL were observed in patients treated with metformin (from 42.7 to 31.5 mg/dl) as well as pioglitazone (from 36.2 to 28.0 mg/dl) but not with sulfonylureas ($p < 0.05$ for both pioglitazone and metformin). Only those randomized to pioglitazone had significant changes in other lipid subfractions, including increases in large LDL particles (from 62.1 to 75.5 mg/dl, $p < 0.001$) as well as increases in

the larger HDL2 fraction (from 11.5 to 15.7 mg/dL, $p < 0.05$). In addition, a study of 50 newly diagnosed patients with diabetes treated with either pioglitazone or metformin determined that pioglitazone significantly reduced the concentration of small LDL-C, but the inter-group difference relative to metformin was not statistically significant [58].

Goldberg and associates randomized 802 diabetic patients in the GLAI study to either pioglitazone ($n = 400$) or rosiglitazone ($n = 402$) in order to analyze differential effects on a variety of lipid parameters using NMR spectroscopy (Figure 4) [59]. The pioglitazone treatment group demonstrated a decrease in LDL particle concentration (from 1394 to 1341 nmol/L; $p = 0.02$) while the rosiglitazone group demonstrated a significant increase (from 1368 to 1488 nmol/L; $p < 0.001$) in the same parameter. While both treatment groups were associated with increases in LDL particle size, the increased particle size was significantly greater in those treated with pioglitazone ($p = 0.005$). In addition, the patients treated with rosiglitazone developed increases in apoB, TG, and nHDL-C, all trends toward a more atherogenic lipoprotein profile. Importantly, there was no significant difference between the two groups in terms of glycemic control as measured by HgbA1c. In a subgroup analysis from the GLAI study of the patients with complete lipoprotein subfractions performed at baseline and at trial completion, 333 patients with diabetes treated with pioglitazone were compared against 325 treated with rosiglitazone therapy [60]. Pioglitazone-treated patients again demonstrated favorable changes to their lipid profile assessed by NMR spectroscopy with a larger decrease in small LDL (least square mean between treatment groups -162.2 nmol/L; $p < 0.001$) and a larger increase in large HDL particle concentration (least square mean between treatment groups 1.0 μ mol/L; $p < 0.001$) relative to rosiglitazone administration. Large LDL particle concentrations increased significantly from baseline in both treatment groups. A small randomized study of nine diabetic patients determined that pioglitazone led to a larger decrease in postprandial small LDL concentration measured by GGE as well as a significant increase in fasting/postprandial large LDL particle concentration relative to rosiglitazone therapy [61].

There is no evidence to date comparing the efficacy of glucagon-like peptide (GLP)-1 or dipeptidyl peptidase (DPP)-4 antagonists in altering lipid subfractions. However, a recent large retrospective analysis compared exenatide, sitagliptin, and insulin therapy and found differential effects on traditional lipid parameters [62]. Specifically, the only therapy to significantly decrease total LDL concentration was exenatide, although all three decreased total cholesterol. In animal studies, augmentation of the incretin response was found to decrease fasting VLDL levels and also to dampen postprandial apolipoprotein-B48 secretion involved in chylomicron formation [63].

Primary lipid-lowering therapies

Statins

Statin therapy has also been proven in a number of studies to have a variety of beneficial effects on the atherogenic diabetic lipid profile (Table 2 & Figure 3). The Collaborative Atorvastatin Diabetes Study (CARDS) was a large multicenter randomized trial which compared atorvastatin 10 mg daily to placebo in patients with diabetes mellitus from the UK and Ireland. Data from the secondary prevention arm was used to compare NMR lipid subfractions in 69 diabetic patients on statin therapy to 53 control patients [64]. Statin therapy was associated with a significant decrease in total LDL particle concentration compared with baseline (from 1572 to 1065 nmol/L; $p < 0.001$). However, this decrease was driven by a decrease in large and medium LDL particles as a non-significant increase in small LDL concentration was observed among those treated with atorvastatin. The patients treated with atorvastatin also demonstrated a significant increase in their large HDL particle concentration (from 3.8 to 5.3 μ mol/L; $p = 0.02$) that was not observed in the placebo group.

Data from the primary prevention arm of CARDS, which randomized 2350 patients with diabetes to atorvastatin or placebo, determined that apoB levels were lower at any given LDL level among the atorvastatin group [65].

The Diabetes Atorvastatin Lipid Intervention cohort, a prospective multicenter Dutch study, randomized diabetic patients to 10 or 80 mg of atorvastatin versus placebo [66]. Both low and high doses of atorvastatin had no significant effect on LDL particle diameter measured by density gradient ultracentrifugation relative to placebo. However, a dose dependent decrease in apoB was described and the amount of cholesterol in all LDL subfractions was decreased in patients treated with atorvastatin. Data from 45 diabetic age/gender matched pairs from the Cholesterol and Recurrent Events trial, a double blind randomized trial of pravastatin 40 mg versus placebo [67], determined that pravastatin decreased the concentration of apoCIII+ lipoproteins determined by ultracentrifugation, a group of apolipoproteins thought to represent atherogenic remnants of VLDL degradation.

Bile acid binding resins

Bile acid binding resins have been demonstrated to produce increases in LDL particle size in nondiabetic patients independent of changes in serum TGs [68]. In a study of 65 diabetic patients randomized to colestevam or placebo on top of baseline oral hypoglycemic therapy, a significant reduction in total LDL particle concentration by NMR spectroscopy was seen in the group treated with colestevam (from 1669 to 1556 nmol/l; $p = 0.006$), a change that was driven mainly by a nonsignificant reduction in small LDL particles (from 1186 to 1145 nmol/l; $p = 0.054$) [69].

Ezetimibe

Ezetimibe, an intestinal cholesterol absorption inhibitor, was studied in a single center randomized trial of healthy, nondiabetic patients both as monotherapy and in combination with simvastatin and was found to increase small, dense LDL particle concentrations and decrease the concentration of large, buoyant LDL particles [70]. However, others analyses have demonstrated ezetimibe to either impart no change in LDL particle size [71] or to decrease the proportion of small, dense LDL [72]. Ezetimibe's effect on lipoprotein subfractions in patients with diabetes has not been reported.

Fibrates

The Diabetes Atherosclerosis Intervention Study (DAIS) demonstrated in a randomized trial of 418 patients with diabetes mellitus that patients given 200 mg of fenofibrate daily had significantly larger LDL particle size by electrophoresis (from 25.66 to 25.07 nm; $p < 0.001$) as well as lower apoB relative to individuals given placebo [73]. In addition, smaller LDL particle size was associated with angiographic worsening on repeat cardiac catheterization performed at the conclusion of the study, with a mean time to repeat catheterization of 39.6 months. Small LDL size was associated with progression of coronary artery disease among the combined study population of fenofibrate and placebo treated patients (Pearson correlation coefficient = -0.16 , $p < 0.01$). However, unlike statins and LDL-C, changes in lipid subfractions with fibrate therapy have not been consistently correlated with improved hard outcomes.

Fish oils

N-3 marine fatty acids are commonly administered to patients with dyslipidemia as they effectively lower TG and nHDL-C levels. Their effects on lipoprotein subclasses have been inconsistent. In a randomized, placebo controlled study of 27 diabetic patients in Norway, those assigned to the fish oil group demonstrated a trend toward increased levels of small (p

= 0.06) and very small LDL ($p = 0.055$) particle concentrations measured by NMR spectroscopy compared with placebo [74]. In addition, patients treated with fish oil also demonstrated a significant decrease in small HDL concentration ($p = 0.004$). By contrast, a trial of fish oil versus placebo in 16 noninsulin dependent diabetic patients using density gradient ultracentrifugation found no change in LDL size pattern over the course of 6 months while diet and hypoglycemic therapy were held constant [75]. The results of both of these studies are in contrast to an earlier study of patients without diabetes mellitus by Baumstark [76] which demonstrated a significant decrease in the small, dense LDL fraction, a reciprocal increase in the large/buoyant LDL fraction and an increase in the protective HDL2b subfraction in seven patients with type IIb hyperlipoproteinemia. It is possible that the effects of fish oil on lipoprotein subfractions in patients with diabetes are disparate from those in nondiabetic patients and will need to be investigated further.

Niacin

Niacin has several beneficial effects on several lipid parameters, including large increases in HDL-C and decreases in TG and nHDL-C, particularly when added to other lipid-lowering agents in many nondiabetic populations. Atorvastatin 10 mg was tested against 3000 mg of immediate release niacin in patients without diabetes mellitus with both interventions significantly reducing small LDL and VLDL particle concentration [77]. Niacin therapy possessed additional advantages including an increase in large LDL particles, large HDL particles, and an overall increase in HDL particle size. Patients treated with 1 g of extended release niacin in a placebo controlled randomized trial of 54 patients in which 12 were diabetic demonstrated significantly lower TG, decreased proportion of small LDL, increased large HDL proportion, and decreased small HDL particle size relative to placebo [78]. Pan *et al.* analyzed the lipid subfractions of 42 diabetic patients during and after niacin therapy by means of electrophoresis, finding a significant decrease in small, dense LDL particle mass (25 mg/dl to 11 mg/dl, $p < 0.01$), an increase in LDL peak diameter (252 angstroms to 262 angstroms, $p < 0.05$), an increase in HDL-C (from 39 to 50 mg/dl; $p < 0.0001$) as well as a corresponding increase in the proportion of cardioprotective HDL2 particles (from 5 to 11 mg/dl; $p < 0.0001$). As observed in studies of niacin in nondiabetic populations, the effects of niacin in diabetic patients are diminished by the high rate of intolerance to the drug [79]. However, there are emerging therapeutic modalities, such as laropiprant which may aid in minimizing the flushing associated with this therapy [80].

Conclusion

Abnormalities in the traditional lipid profile provide some insight into the increased CVD burden of patients with diabetes mellitus but do not fully explain their high risk status. Several studies have demonstrated variability in lipoprotein particle composition even in patients with grossly similar total cholesterol measurements, suggesting that the lipid manifestations of diabetes mellitus may best be observed by analyzing lipoprotein subfractions [21,22]. Interestingly, the atherogenic subfraction profile (Pattern B) seems to manifest even prior to the development of diabetes mellitus which suggests an abnormality in lipid function and composition that is independent of hyperglycemia. Advanced lipoprotein testing can thus provide a means to explain the incremental CVD risk of diabetes mellitus, as evidenced by the recent landmark MESA study highlighting the marked increase in discordant lipid fractions in patients with MetSyn and diabetes and failure of LDL-C to predict incident cardiovascular events in the discordant population.

Whether measurement of lipoprotein subfractions in patients with diabetes or MetSyn will improve clinical utility in prognosis or assessing response to therapies remains to be seen. Based on current guidelines, LDL-C and nHDL-C remain the primary targets in reducing lipid-based risk in patients with diabetes or MetSyn [16]. Measuring apoB and lipid

subfractions may be useful once LDL-C and nHDL-C targets are achieved given the marked increase in discordant lipid fractions in these populations. Statins remain an integral and invaluable component of the pharmacologic management of dyslipidemia, and large randomized trials such as the Heart Protection Study [81] have demonstrated a reduction in major cardiovascular events in those patients with diabetes mellitus with and without established CVD. Several studies analyzed in this review found a favorable change in the lipid subfractions of diabetic patients treated with relatively low doses of statins which suggest benefits of statin therapy beyond simple LDL-C reduction. However, there are a number of other therapeutic interventions which are relatively underused and can also elicit favorable changes in lipid subfractions in patients with diabetes mellitus. Niacin and fenofibrate, for example, have been demonstrated to significantly improve LDL particle size in this population. Furthermore, agents designed to achieve glucose control in patients with diabetes such as pioglitazone and metformin also achieved beneficial changes within the lipid profile. These pleiotropic effects, which frequently occurred independently of glycemic control, could warrant further utilization of these agents in patients with diabetes mellitus or MetSyn, particularly those at markedly higher risk for CVD. It remains to be seen whether these beneficial changes in lipid subfractions associated with nonstatin pharmacologic therapies actually translate into improved clinical outcomes.

The precise clinical role of advanced lipoprotein testing in the general population is presently unclear. The lack of standardization and the absence of a 'gold standard' technique by which to compare the various technologies have slowed its adaptation and utilization. Advanced lipoprotein testing has provided great insight into a potential means by which diabetic patients develop additional risk for CVD. However, the clinical applicability of this technology remains controversial. Analysis of lipid subfractions may offer a means of risk stratification and identification of patients that may benefit from multidrug pharmacotherapy. Development of efficient and low-cost testing for small dense LDL (Pattern B) will facilitate further research and clinical use. The ultimate test of the applicability of advanced lipoprotein testing is whether it will promote the initiation of beneficial therapeutic changes in patients. To date, no large, randomized outcome studies have been performed to answer this question.

Future perspective

The prevalence of diabetes mellitus and the MetSyn will only continue to increase over the coming years. The rapid growth of this high risk population will require aggressive risk factor modification. Diabetic dyslipidemia, while partially explained by characteristic cholesterol abnormalities, also manifests as abnormalities of lipoprotein particles out of proportion to traditional cholesterol measurements. Measurement of these advanced lipoprotein parameters have allowed for a more nuanced assessment of lipid abnormalities in diabetes mellitus. As our knowledge regarding diabetic dyslipidemia grows, possibilities of novel therapeutic interventions and more efficient risk stratification will likely emerge. The lack of consistency across and within these technologies nevertheless is the most prominent limiting factor in its broader utilization. There is a need for additional comparative studies of the lipid subfraction methodologies that will facilitate standardization in this emerging field as well as validation for improved clinical utility in this high risk population.

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Executive summary

- The prevalence of diabetes mellitus and the metabolic syndrome is increasing.
- Both diabetes mellitus and the metabolic syndrome carry an increased risk of cardiovascular disease.
- Novel technologies allow for advanced lipoprotein analysis such as nuclear magnetic resonance, electrophoresis and ultracentrifugation, though there is a lack of standardization.
- Small, dense LDL (Pattern B phenotype) and elevated apolipoprotein B (apoB) have been associated with an increased risk of cardiovascular disease.

The atherogenic lipid profile in insulin resistant states

- The diabetic lipid profile possesses characteristic abnormalities including a prominence of small, dense LDL and small HDL particles as well as an elevation in apoB and triglycerides.
- These lipoprotein particle abnormalities persist even in patients with normal LDL-C and HDL-C.
- The MESA study determined that LDL particle (LDL-P) concentration is a better predictor of incident cardiovascular disease when LDL-P and LDL-C concentrations are discordant (LDL-P > LDL-C).

Therapies & lipid subfraction changes in patients with diabetes

- Glycemic control
 - Enhanced glycemic control with exercise and diet control improves atherogenic profile in diabetes mellitus and increases the probability of changing from a Pattern B to a Pattern A (large, buoyant LDL) phenotype.
 - Ability to change lipoprotein particle size is greatest in those with a Pattern B phenotype at baseline.
 - Pioglitazone produces favorable changes in the lipid abnormalities of diabetes mellitus relative to rosiglitazone and troglitazone.
 - Incretin augmentation has not been studied in this context.
- Lipid lowering
 - Statins have been extensively studied in this context and have significantly decreased atherogenic lipoproteins in diabetes mellitus, with atorvastatin being the most studied statin.
 - Colesevelam and fenofibrate increase LDL particle size. The effect of ezetimibe on lipid subfractions has not been assessed in patients with diabetes mellitus.
 - Niacin, though it was only studied in one trial, significantly decreased small LDL-P concentration.

Conclusion

- Advanced lipoprotein parameters can be utilized after LDL-C and non-HDL cholesterol targets are achieved in patients with the metabolic syndrome and diabetes mellitus.
- The clinical applicability of this technology is unclear, but the recent MESA study indicates that LDL-P concentration may be more useful than LDL-C in metabolic syndrome and diabetes mellitus as a predictor of cardiovascular disease risk.

	← Increasing atherogenicity						
	Small, dense LDL			Large, buoyant LDL			
Ultracentrifugation	LDL4	LDL3		LDL2	LDL1		
Gradient gel electrophoresis	IVb	IVa	IIIb	IIIa	IIb	IIa	I
Nuclear magnetic resonance	LDL1			LDL2		LDL3	
	Small, dense HDL			Large, buoyant HDL			
Ultracentrifugation	HDL3			HDL2			
Gradient gel electrophoresis	HDL3c	HDL3b		HDL3a	HDL2a	HDL2b	
Nuclear magnetic resonance	H1	H2		H3	H4	H5	

Figure 1. LDL and HDL subfractions reported by the common commercial lipid subfraction methodologies

While both LDL-C concentrations are equivalent, the distribution of LDL-C differs depending on LDL particle concentrations. An increased percentage of small, dense LDL particles is described as the Pattern B phenotype and has been associated with an increased risk of coronary artery disease. Ultracentrifugation subfraction parameters are from the Vertical Auto Profile (Atherotech, Birmingham, AL, USA), gradient gel electrophoresis parameters are from Berkeley Heart Lab (South San Francisco, CA, USA), and nuclear magnetic resonance spectroscopy parameters are from LipoScience (Raleigh, NC, USA).

Figure 2. Variability in LDL particle composition despite equivalent LDL-C levels

Figure 3. Changes in lipid subfractions by lipid- and glucose-lowering therapies in patients with diabetes

Mean percentage changes are reported when multiple studies for any particular therapy were available. Data for all lipid parameters was not available for each therapy. apoB: Apolipoprotein B; MET: Metformin; PIO: Pioglitazone; ROS: Rosiglitazone; sdLDL: Small, dense LDL; SU: Sulfonylurea; TLC: Therapeutic lifestyle changes.

Figure 4. Variable changes in lipid subfractions among subjects treated with pioglitazone and rosiglitazone despite equivalent glycemic control in the GLAI Study

Despite no significant differences in glycemic control, considerable variability is observed in the lipid subfractions of patients receiving PIO versus ROS in the GLAI Study [58,59]. HgbA1c: Hemoglobin A1c; PIO: Pioglitazone; ROS: Rosiglitazone.

Table 1

The most common lipid subfraction methods.

Lipoprotein subfraction method	Commercial assay	Reported lipid parameters	Ref.
Density gradient ultracentrifugation			
Ultracentrifugation is the oldest of the lipid subfraction technologies, initially described the 1950s and 1960s as a method to fractionate lipoproteins. Particles are separated according to size and density properties.	Vertical Auto Profile (Atherotech, Birmingham, AL, USA)	VLDL1,2 = buoyant VLDL3 = dense IDL LDL1,2 = large/buoyant LDL3,4 = small/dense HDL2 = large/buoyant HDL3 = small/dense apoB apoA1 Lp(a)	[10,82]
Gradient gel electrophoresis			
This technology is commonly used in various commercial and academic applications. It was described by Krauss and Burke in 1982 and is used extensively in a variety of laboratories globally. Size and charge variability are the key determinants of particle differentiation.	Berkeley GGE (Berkeley Heart Lab, South San Francisco, CA, USA)	LDL I, IIa, IIb = large/buoyant LDL IIIa, IIb, IVa, IVb = small/dense HDL2b = large/buoyant HDL2a, 3a, 3b, 3c = small/dense apoB Lp(a) In addition to lipids, Berkeley HeartLab reports: Homocysteine CRP apoE genotype	[11,83]
NMR spectroscopy			
NMR as a lipid fractionation technique was described by Otvos, which separates lipoprotein particles based on differing methyl group NMR signals.	LipoScience NMR (LipoScience, Raleigh, NC, USA)	VLDL V1-V6, V1 = small/dense LDL1 = small/dense LDL2 = intermediate LDL3 = large/buoyant H1 = small/dense H5 = large/buoyant LDL size HDL size VLDL size LP-IR	[12]

apoA1: Apolipoprotein A1; apoB: Apolipoprotein B; CRP: C-reactive protein; Lp(a): Lipoprotein A; LP-IR: Lipoprotein insulin resistance; NMR: Nuclear magnetic resonance.

Table 2

Summary of observational studies of lipid subfractions in diabetes mellitus and the metabolic syndrome.

Study (year)	Patients (n)	Country	Subfraction method	Results [†]	Ref.
Feingold <i>et al.</i> (1992)	29 DM2, 87 control	USA	Gradient gel electrophoresis	LDL phenotype comparison: less Pattern A in DM2 (47 vs 28%, $p = 0.025$), increased Pattern B in DM2 (24 vs 52%, $p < 0.025$)	[28]
Selby <i>et al.</i> (1993)	704 female twins	USA (Kaiser Permanente Women Twins Study)	Gradient gel electrophoresis	↑ TG, waist:hip ratio, fasting insulin, 2 h postload insulin, 2 h postload glucose with increased probability of Pattern B, ↓ HDL associated with increased likelihood of Pattern B in female twins	[32]
Haffner <i>et al.</i> (1994)	95 DM2, 371 nondiabetic controls	USA (San Antonio Heart Study)	Analytical ultracentrifugation	LDL size ↓ in both male and female patients with DM2, relationship explained by HDL and TG in males	[30]
Syvanne <i>et al.</i> (1995)	50 consecutive patients with DM2 and CAD	Finland	Gradient gel electrophoresis	HDL2a ↑ in DM2 patients, other subfractions NS difference	[36]
Siegel <i>et al.</i> (1996)	1878 male controls (120 male DM2); 1879 female controls (54 female DM2)	USA (Framingham Heart Study)	Gradient gel electrophoresis	Pattern B in males with DM2: 49.2 vs 32.5% ($p < 0.05$), Pattern B in females with DM2: 10.7 vs 40.3% ($p < 0.001$), apoB significantly ↑ in females with DM2	[27]
Friedlander <i>et al.</i> (2000)	200 male, 190 female without DM2	Israel	Gradient gel electrophoresis	↑ TG, fasting insulin, 2 h postload insulin positively related to LDL particle size, HDL negatively related to LDL particle size	[31]
Garvey <i>et al.</i> (2003)	46 DM2, 56 insulin sensitive, 46 insulin resistant	USA	NMR	↑ Total LDL, large LDL, intermediate LDL and small LDL in patients with DM	[84]
Tan <i>et al.</i> (2001)	93 consecutive DM2 male	Singapore	Gradient gel electrophoresis	DM2 patients with ↑ small LDL and ↓ large LDL even with mean A1c of 6.6%	[29]
Kathiresan <i>et al.</i> (2006)	376 males and 265 females with MetSyn against 1028 male and 1324 female controls	USA (Framingham Heart Study)	NMR	MetSyn ↑ total LDL-P, small LDL-P, apoB ↓ LDL size, HDL size, large LDL, large HDL	[46]
Festa <i>et al.</i> (2005)	830 nondiabetic patients at baseline, 130 converted to DM2	USA (Insulin Resistance Atherosclerosis Study)	NMR	↑ Total LDL particles, small LDL, small HDL, VLDL size and ↓ LDL/HDL particle size in patients who became diabetic	[39]
Gazi <i>et al.</i> (2006)	105 with MetSyn, 70 controls	Greece	Polyacrylamide gel electrophoresis	MetSyn ↑ cholesterol concentration in dense LDL and VLDL ↓ LDL mean particle size and peak particle diameter	[47]
Rizzo <i>et al.</i> (2009)	124 with MetSyn, 102 controls	Italy	Gradient gel electrophoresis	LDL particle size, low HDL-C predictors of cardio/cerebrovascular risk at 2 years	[48]

Study (year)	Patients (n)	Country	Subfraction method	Results [‡]	Ref.
Orvos <i>et al.</i> (2011)	6814 from community-based cohort	USA (Multi-Ethnic Study of Atherosclerosis)	NMR	↑ Prevalence of DM2 and MetSyn in discordant LDL-P > LDL-C; both LDL-P and LDL-C associated with incident CVD in concordant, only LDL-P associated with CVD in discordant	[50]

CVD: Cardiovascular disease; DM2: Type 2 diabetes mellitus; LDL-P: LDL particle; MetSyn: Metabolic syndrome; NMR: Nuclear magnetic resonance; NS: Not significant; TG: Triglyceride.

[‡] All changes noted are statistically significant unless marked NS.

Table 3

Discordant LDL-C and LDL-P and cardiovascular disease from the Multi-Ethnic Study of Atherosclerosis.

	LDL-P > LDL-C (n = 1407) [†]	LDL-P = LDL-C (n = 2775)	LDL-P < LDL-C (n = 1416) [†]
DM (%)	19	14	9
MetSyn (%)	54	33	16
LDL-P (nmol/l)	1372 ± 240	1249 ± 395	1117 ± 201
LDL-C (mg/dl)	104 ± 20	117 ± 37	130 ± 23
LDL size (nm)	20.3 ± 0.5	20.7 ± 0.5	21.1 ± 0.4
HOMA-IR	2.4 ± 2.8	1.8 ± 1.8	1.5 ± 1.8

[†] Significant difference compared with the concordant subgroup, $p < 0.0001$. HOMA-IR is a measure of insulin resistance. LDL size was measured with nuclear magnetic resonance spectroscopy. Adapted from [50].

Table 4

Discordant LDL-C and LDL-P and cardiovascular disease from the Multi-Ethnic Study of Atherosclerosis.

Predictor of incident cardiovascular events	Hazard ratio (95% CI)
<i>LDL-P & LDL-C concordant</i>	
LDL-C	1.27 (1.12–1.44); p = 0.0003
LDL-P	1.27 (1.12–1.44); p = 0.0002
<i>LDL-P & LDL-C discordant</i>	
LDL-C	1.07 (0.88–1.30); p = 0.52
LDL-P	1.45 (1.19–1.78); p = 0.0003

Adapted from [50].

Table 5

Summary of trials of lipid subfraction changes in response to various lipid- and glucose-lowering therapies in diabetes mellitus.

Author	Patients, country	Intervention	Study type	Subfraction method	Results [†]	Ref.
Therapeutic lifestyle changes						
Halle <i>et al.</i> (1998)	34 DM2, Germany	2200 kcal/week exercise; 1000 kcal/day diet	Prospective, longitudinal	Density gradient ultracentrifugation	TLC ↑ large LDL, ↓ small LDL, ↓ apoB	[51]
Wagner <i>et al.</i> (2003)	33 poorly controlled DM2, Spain	TLC, exercise, oral hypoglycemic agents	Prospective, longitudinal	Gradient gel electrophoresis	↓ LDL, ↑ HDL, ↓ apoB, ↓ Pattern B, ↑ LDL particle size in Pattern B, no LDL particle size change in Pattern A	[52]
Insulin						
Caixas <i>et al.</i> (1997)	37 DM1, 33 DM2, poorly controlled, 33 control, Spain	Intensive insulin therapy	Prospective, longitudinal	Ultracentrifugation	↓ Pattern B after glycemic control in NIDDM, larger ↓ in HgbA1c in patients that converted Pattern B ⇒ A	[53]
Rivellese <i>et al.</i> (2000)	9 DM2, Italy	Insulin vs glibenclamide	Randomized	Density gradient ultracentrifugation	Insulin ↓ amount/proportion of small LDL and ↑ large LDL	[54]
MET						
Ohira <i>et al.</i> (2007)	28 DM2, Japan	MET 500 mg BID	Prospective, longitudinal	Polyacrylamide gel disc electrophoresis	↑ LDL particle size independent of improvement in Hgb A1c and fasting blood glucose	[55]
TZD						
Bavirti <i>et al.</i> (2003)	10 DM2, USA	Troglitazone	Prospective, longitudinal	NMR	↓ small LDL (NS), large HDL ↑ LDL particle size, large LDL, small HDL	[56]
Lawrence <i>et al.</i> (2004)	67 DM2, UK	MET 500 mg BID vs PIO 30 daily vs glitazide 80 mg daily	Randomized, open label, parallel	Density gradient ultracentrifugation	MET ↓ small LDL, apoB PIO ↓ small LDL ↑ HDL2, large LDL. SU ⇒ NS change	[57]
Goldberg <i>et al.</i> (2005)	GLAI, 369 PIO, 366 ROS, USA/ Puerto Rico/ Mexico/ Colombia	PIO 45 mg vs ROS 4 mg BID	Randomized, open label, parallel	NMR	PIO ↓ LDL particle conc, PIO with greater increase in particle size than ROS, ROS ↑ LDL particle concentration	[59]
Deeg <i>et al.</i> (2007)	GLAI, 369 PIO, 366 ROS, USA/ Puerto Rico/ Mexico/ Colombia	PIO 30 mg vs ROS 4 mg BID	Randomized, open label, parallel	NMR	PIO ↑ large HDL ↓ small LDL to greater extent than ROS, both ↑ large LDL	[60]
Nakano <i>et al.</i> (2010)	50 DM2, newly diagnosed, Japan	MET 500 mg/ 1000 mg BID vs	Randomized, open label, parallel	HPLC	PIO ↓ very small LDL, large VLDL,	[58]

Author	Patients, country	Intervention	Study type	Subfraction method	Results [†]	Ref.
Incretin						
		PIO 15 mg daily			MET ↓ very small LDL	
Horton <i>et al.</i> (2010)	6280 exenatide, 3861 sitagliptin, 3239 insulin, USA	Exenatide vs sitagliptin vs insulin	Retrospective	None	Exenatide ↓ LDL, exenatide/sitagliptin ↓ TG, Insulin (NS)/exenatide (NS) ↓ HDL	[62]
Statin						
Soedamah-Muthu <i>et al.</i> (2003)	122 DM2, UK/Ireland	ATORVA 10 mg vs placebo	Randomized, double blind	NMR	ATORVA ↓ large/medium LDL, small LDL (NS); LDL/VLDL particle number ↑ large HDL	[64]
Lee <i>et al.</i> (2003)	CRE, 90 DM2, USA	PRAVA 40 mg vs placebo	Randomized, prospective	Ultracentrifugation	PRAVA ↓ IDL + LDL CIII+	[67]
Charlton-Menys <i>et al.</i> (2009)	2350 DM2, UK/Ireland	ATORVA 10 mg vs placebo	Randomized, double blind	NMR	ATORVA ↓ apoB at all LDL levels	[65]
Kappelle <i>et al.</i> (2010)	DALI, 185 DM2, Netherlands	Placebo vs 10 mg ATORVA vs 80 mg ATORVA	Randomized, prospective	Density gradient ultracentrifugation	ATORVA ↓ cholesterol in all LDL subfractions, no change mean particle size, dose dependent ↓ apoB	[66]
Bile acid binding resin						
Rosenson <i>et al.</i> (2006)	65 DM2, USA	COLE 3.75 g/day vs placebo	Randomized, prospective	NMR	COLE ↓ total LDL particle conc., small LDL (p = 0.054)	[68]
Fish oil						
Patti <i>et al.</i> (1999)	16 DM2, Italy	Fish oil vs placebo	Randomized	Density gradient ultracentrifugation	Fish oil tended ↓ all VLDL subfractions, no significant change LDL subfractions or LDL particle size	[75]
Mostad <i>et al.</i> (2008)	27 DM2, Norway	Corn oil vs fish oil	Randomized, prospective	NMR	Fish oil tended ↑ small/very small LDL (NS), ↓ small HDL	[74]
Niacin						
Pan <i>et al.</i> (2002)	65 DM2, USA	Niacin 100 mg TID vs placebo	Retrospective	Polyacrylamide gel electrophoresis	Niacin ↑ HDL2, LDL particle size ↓ small LDL	[79]
FENO						
Vakkilainen <i>et al.</i> (2003)	418 DM2, Finland/Sweden/France/Canada	FENO 200 mg daily vs placebo	Randomized, prospective	Polyacrylamide gel electrophoresis	FENO ↑ LDL particle size ↓ apoB	[73]

ATORVA: Atorvastatin; BID: Twice daily; CIII: Apolipoprotein-CIII; COLE: Colesevelam; Conc.: Concentration; DM1: Type 1 diabetes mellitus; DM2: Type 2 diabetes mellitus; FENO: Fenofibrate; HgbA1c: Hemoglobin A1c; MET: Metformin; NIDDM: Noninsulin-dependent diabetes mellitus; NMR: Nuclear magnetic resonance; NS: Not significant; PIO: Pioglitazone; PRAVA: Pravastatin; ROS: Rosiglitazone; SU: Sulfonylurea; TLC: Therapeutic lifestyle changes; TZD: Thiazolidinediones.

[†] All changes noted are statistically significant unless marked NS.