Lifestyle and Metformin Treatment Favorably Influence Lipoprotein Subfraction Distribution in the Diabetes Prevention Program

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Context: Although intensive lifestyle change (ILS) and metformin reduce diabetes incidence in subjects with impaired glucose tolerance (IGT), their effects on lipoprotein subfractions have not been studied.

Objective: The objective of the study was to characterize the effects of ILS and metformin vs placebo interventions on lipoprotein subfractions in the Diabetes Prevention Program.

Design: This was a randomized clinical trial, testing the effects of ILS, metformin, and placebo on diabetes development in subjects with IGT.

Participants: Selected individuals with IGT randomized in the Diabetes Prevention Program participated in the study.

Interventions: Interventions included randomization to metformin 850 mg or placebo twice daily or ILS aimed at a 7% weight loss using a low-fat diet with increased physical activity.

Main Outcome Measures: Lipoprotein subfraction size, density, and concentration measured by magnetic resonance and density gradient ultracentrifugation at baseline and 1 year were measured.

Results: ILS decreased large and buoyant very low-density lipoprotein, small and dense low-density lipoprotein (LDL), and small high-density lipoprotein (HDL) and raised large HDL. Metformin modestly reduced small and dense LDL and raised small and large HDL. Change in insulin resistance largely accounted for the intervention-associated decreases in large very low-density lipoprotein, whereas changes in body mass index (BMI) and adiponectin were strongly associated with changes in LDL. Baseline and a change in adiponectin were related to change in large HDL, and BMI change associated with small HDL change. The effect of metformin to increase small HDL was independent of adiponectin, BMI, and insulin resistance.

Conclusion: ILS and metformin treatment have favorable effects on lipoprotein subfractions that are primarily mediated by intervention-related changes in insulin resistance, BMI, and adiponectin. Interventions that slow the development of diabetes may also retard the progression of atherosclerosis. **(*J Clin Endocrinol Metab* 98: 3989–3998, 2013)**

The dyslipidemia associated with insulin resistance, characterized by elevated triglyceride and reduced high-density lipoprotein cholesterol (HDL-C) levels, contributes to the elevated cardiovascular risk in type 2 diabetes. Delineating the modifications of lipoprotein subfractions that underlie this dyslipidemia provides an opportunity to better understand the atherogenic determinants of dyslipidemia in these subjects. Studies to date have observed an increase in large very low-density lipoprotein (VLDL) and small, dense low-density lipoprotein

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Abbreviations: BMI, body mass index; DGU, density gradient ultracentrifugation; DPP, Diabetes Prevention Program; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; IGT, impaired glucose tolerance; ILS, intensive lifestyle change; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; MET, metabolic equivalent; NMR, nuclear magnetic resonance; P, particle concentration; VLDL, very low-density lipoprotein.

(LDL) particles in the dyslipidemia of type 2 diabetes (1). Changes in high-density lipoprotein (HDL) subfractions have been less well documented.

Interventions that delay development of type 2 diabetes in subjects with impaired glucose tolerance (IGT) may also modify associated risk factors for the cardiovascular complications of this disease (2, 3). Although reports have demonstrated that lifestyle intervention or medications that slow diabetes development modify lipid levels in subjects with IGT (2), as yet there are no reports of the effects of these interventions on lipoprotein subfractions in this setting. We report here the effect of lifestyle change and metformin treatment on lipoprotein subfractions measured using two complementary methodologies, namely nuclear magnetic resonance (NMR) and density gradient ultracentrifugation (DGU) in participants with IGT in the Diabetes Prevention Program (DPP). In addition we explore the importance of anthropometric, metabolic, and lifestyle variables in explaining these intervention effects.

Materials and Methods

Study participants and procedures

The design of the DPP has been reported elsewhere (1). In brief, selection criteria included the following: age of 25 years or older, body mass index (BMI) of 24 kg/m^2 or greater ($\geq$22 kg/m^2 in Asian Americans), fasting plasma glucose levels between 95 and 125 mg/dL and 2-hour postload glucose of 140–199 mg/dL. Exclusion criteria included taking medications known to alter glucose tolerance, a cardiovascular disease event in the prior 6 months, or illnesses that could seriously reduce ability to participate.

Participants were randomly assigned to one of three interventions: metformin 850 mg or placebo twice daily or an intensive program of lifestyle modification (ILS). Treatment assignments were stratified according to clinical center and double blinded for the metformin and placebo groups. The goals of the ILS were to achieve and maintain a weight reduction of at least 7% of initial body weight through the consumption of a low-calorie, low-fat diet and to engage in moderate physical activity for at least 150 min/wk. Diabetes was diagnosed on the basis of an annual oral glucose tolerance test or a semiannual fasting plasma glucose test according to American Diabetes Association criteria. The diagnosis required confirmation by a second test, usually within 6 weeks.

The current report includes a subset of the 3234 randomized participants who had appropriate blood samples stored at −70°C and available from study visits at baseline (n = 2023), with 1645 paired samples (553 placebo, 558 metformin, and 534 ILS) available for the analysis of the changes with intervention at 1 year. The availability of samples differed by age, race, and sex (all $P < .001$) from the full cohort but not by treatment group ($P = .86$). Although the results are not generalizable to the randomized cohort, treatment group comparisons remain valid. In a multivariate logistic regression model to assess whether metabolic parameters are associated with the missing outcomes, we failed to detect any association between baseline or changes in BMI, homeostatic model assessment of insulin resistance (HOMA-IR), HDL-C, triglycerides and fasting glucose, and the missing mechanism. Thus, the assumption of missing at random seems reasonable. Written informed consent was obtained from all participants, consistent with the Declaration of Helsinki and the guidelines of each center's institutional review board.

Clinical and metabolic variables

Standardized interviewer-administered questionnaires were used to obtain demographic and clinical data. Blood pressure and anthropometrics were measured using standard techniques. Diet information was collected by interview at baseline and at year 1 using a modified Block food-frequency questionnaire (4). Total metabolic equivalent (MET) hours per week of physical activity was assessed by the 1-year recall Modifiable Activity Questionnaire (5). Glucose, insulin, and lipid profile measurements were performed at the Central Biochemistry Laboratory (Northwest Lipid Research Laboratories, University of Washington, Seattle, Washington) as previously reported (1). The HOMA-IR was calculated as fasting insulin (microunits per milliliter) × fasting glucose (millimoles per liter)/22.5 (6). The total circulating adiponectin was measured using a latex particle-enhanced turbidimetric assay (Otsuka Pharmaceutical) (7).

Lipoprotein subclass particle concentrations and average VLDL, LDL, and HDL particle diameters were measured by NMR spectroscopy at LipoScience, Inc using the LipoProfile-3 algorithm (8, 9). Weighted-average VLDL, LDL, and HDL particle sizes (in nanometer diameter units) are computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its NMR signal (9). The following 8 subclass categories were investigated: 1) large VLDL (including chylomicrons, if present) (>60 nm); 2) medium VLDL (42–60 nm); 3) small VLDL (29–42 nm); 4) large LDL (20.5–23.0 nm); 5) small LDL (18.0–20.5 nm); 6) large HDL (9.4–14.0 nm); 7) medium HDL (8.2–9.4 nm); and 8) small HDL (7.3–8.2 nm). VLDL and LDL subclass particle concentrations are expressed in units of nanometers per liter and HDL subclasses in micrometers per liter. Summation of the subclass levels provides total VLDL, LDL, and HDL particle concentrations. Coefficients of variation were less than 10% for all measurements.

Lipoprotein density distribution was determined by nonequilibrium DGU using a modification of a previously described technique and a vertical rotor (Beckman VTI-65; Beckman Instruments) (10). Cholesterol (milligrams per deciliter) was measured as an absolute value in each fraction. HDL is typically located in fractions 0–6, LDL in fractions 7–18, intermediate density lipoprotein (IDL) in fractions 19–30, and VLDL in fractions 31–38.

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Table 1. Clinical and Metabolic Measurements by Treatment Group at Baseline and After 1 Year of Intervention

	Baseline			Year 1		
	Placebo	Metformin	Lifestyle	Placebo	Metformin	Lifestyle
BMI, kg/m^2	33.7 ± 6.6	33.7 ± 6.5	33.5 ± 6.4	33.7 ± 6.9	32.5 ± 6.5[a]	30.8 ± 6.0[a,b]
Waist, cm	104 ± 14	105 ± 15	105 ± 14	104 ± 14	102 ± 14	98 ± 14[a,b]
0′ glucose, mmol/L	5.9 ± 0.4	5.9 ± 0.4	5.9 ± 0.4	5.94 ± 0.78	5.66 ± 0.56[a]	5.61 ± 0.56[a]
HbA1c, %	5.93 ± 0.48	5.93 ± 0.50	5.91 ± 0.52	6.00 ± 0.59	5.91 ± 0.48[a]	5.79 ± 0.48[a,b]
LDL-C, mmol/L	3.24 ± 0.85	3.24 ± 0.83	3.21 ± 0.83	3.16 ± 0.80	3.08 ± 0.80	3.06 ± 0.75
Non-HDL-C, mmol/L	4.09 ± 0.93	4.07 ± 0.91	4.07 ± 0.93	4.01 ± 0.85	3.91 ± 0.88	3.78 ± 0.83[a]
Triglycerides, mmol/L	1.7 [1.2, 2.4]	1.6 [1.1, 2.3]	1.6 [1.1, 2.3]	1.55 [1.12, 2.16]	1.53 [1.07, 2.11]	1.32 [0.94, 1.89][a,b]
HDL-C, mmol/L	1.17 ± 0.10	1.20 ± 0.30	1.20 ± 0.33	1.17 ± 0.31	1.22 ± 0.31[a]	1.24 ± 0.34[a]
Lipid medications, %	44 (6.5%)	43 (6.4%)	28 (4.2%)	70 (12.7%)	57 (10.2%)	42 (7.9%)[a]
Physical activity, MET h/wk	10 [4, 22]	11 [4, 22]	6 [4, 21]	11 [4, 23]	12 [6, 24]	17 [10, 28][a,b]
Saturated fat, g	28 ± 19	28 ± 18	28 ± 19	23.5 ± 15.2	22.6 ± 13.0	16.4 ± 10.0[a,b]
HOMA-IR	6.1 [4.1, 8.6]	6.2 [4.2, 9.0]	6.0 [4.2, 8.7]	5.8 [4.3, 9.1]	5.1 [3.4, 7.2][a]	4.3 [2.9, 6.4][a]
Adiponectin, μg/mL	7.9 ± 3.4	8.2 ± 3.6	8.1 ± 3.7	8.0 ± 3.4	8.5 ± 3.9	9.0 ± 3.9[a,b]

Abbreviation: HbA1c, glycosylated hemoglobin. Baseline and year 1 data are presented as mean (SD), median [interquartile range], and number (percentage) as appropriate.

[a] Treatment group comparisons at baseline resulted in $P < .01$ vs placebo.

[b] Treatment group comparisons at year 1 resulted in $P < .01$ vs metformin.

Data analysis

This analysis is based on data and samples collected at baseline and at the end of year 1 using SAS 9.2 (SAS Institute). Due to the multiplicity of testing, we restricted tests for subfractions of HDL and VLDL particles to minimize a type 1 error. Differences among treatment groups were assessed by the χ^2 test for categorical covariates and ANOVA or the median test (as appropriate) for continuous covariates with the significance level set at $P = .01$ using unadjusted P values. The primary analyses of changes in lipoprotein subfractions were assessed using an analysis of covariance with an adjustment for baseline value and the use of lipid-lowering medications, and P values were adjusted for the 3 pairwise comparisons. Spearman correlations were used to describe the bivariate relationships among the lipoprotein subfractions, anthropometric and metabolic variables with adjustment for age at randomization, sex, and race/ethnicity. Multiple regression models were used to examine which changes in key metabolic, anthropometric, and lifestyle variables accompanied the change in lipoprotein subfraction from baseline to year 1. Change in lipoprotein subfraction was the dependent variable with the following independent covariates: treatment assignment, age at baseline, sex, race, and baseline and change in metabolic variables. Separate models were constructed for each lipoprotein subfraction. Based on the baseline univariate correlations, HOMA-IR, BMI, and adiponectin were identified as possible determinants of the intervention-related changes in lipoprotein subfractions for the regression models. Triglyceride was not included as a covariate because it is a lipoprotein subfraction surrogate. All regression models were adjusted for the use of lipid-modifying medications.

Results

Clinical and metabolic measurements

Table 1 summarizes the key clinical and metabolic assessments at baseline and after 1 year of intervention. There were no differences among the three intervention groups at baseline. At 1 year there were significant differences in anthropometric, glycemia, HOMA-IR, adiponectin, physical activity, and saturated fat intake measures as well as the use of lipid-lowering medications between the three groups. Triglyceride levels decreased in ILS only, whereas HDL-C increased in both the ILS and metformin groups. LDL cholesterol (LDL-C) did not change in any of the groups.

Lipoprotein subfractions by NMR

The unadjusted mean levels of lipoprotein size and particle concentration (P) at baseline and 1 year are shown in Table 2. There were no differences in baseline values between the three groups. At 1 year large VLDL-P was lower and VLDL size was smaller in the ILS vs the placebo and metformin groups ($P < .01$). LDL size and large LDL-P were higher and small LDL-P was lower in both metformin and ILS after 1 year compared with the placebo (all $P < .01$), with the ILS values being greater (all $P < .01$) than in the metformin group. Large HDL-P and HDL size were greater and small HDL-P was lower in the ILS compared with the placebo and metformin groups, with large HDL-P higher in the metformin than the placebo groups (all $P < .01$).

Figure 1 demonstrates the change from baseline in these parameters adjusted for baseline levels and lipid-lowering medications. There was a reduction in large VLDL-P and VLDL size in the ILS vs the metformin and placebo groups (Figure 1, A and D; $P < .01$). Despite the absence of a change in LDL-C, total LDL-P was reduced in the ILS group due to a fall in small LDL-P, offset to some degree by an increase in large LDL-P (Figure 1B; all $P < .01$).

Table 2. Lipoprotein Subfractions Measured by NMR by Treatment Group at Baseline and After 1 Year of Intervention

	Baseline			Year 1		
	Placebo	**Metformin**	**Lifestyle**	**Placebo**	**Metformin**	**Lifestyle**
VLDL-P						
Size, nm	53 ± 8	54 ± 8	53 ± 8	53 ± 8	53 ± 8	51 ± 8[a,b]
Small, nmol/L	31 ± 18	30 ± 17	30 ± 17	31 ± 18	31 ± 17	29 ± 17
Medium, nmol/L	30 ± 20	29 ± 20	30 ± 21	31 ± 20	30 ± 20	28 ± 20
Large, nmol/L	8.0 ± 7.3	8.1 ± 7.1	8.1 ± 7.8	8.1 ± 8.1	7.5 ± 7.3	6.1 ± 6.5[a,b]
LDL-P						
Size, nm	20.5 ± 0.6	20.5 ± 0.6	20.5 ± 0.6	20.5 ± 0.6	20.5 ± 0.6	20.7 ± 0.6[a,b]
Total, nmol/L	1398 ± 381	1383 ± 389	1368 ± 369	1344 ± 358	1291 ± 345	1236 ± 344[a,b]
Small, nmol/L	806 ± 405	793 ± 394	780 ± 400	784 ± 392	711 ± 354[a]	626 ± 396[a]
Large, nmol/L	422 ± 262	423 ± 255	426 ± 268	407 ± 260	435 ± 270	460 ± 257[a]
HDL-P						
Size, nm	8.9 ± 0.4	8.9 ± 0.4	8.9 ± 0.4	8.9 ± 0.4	8.9 ± 0.4	9.0 ± 0.4[a,b]
Total, μmol/L	35 ± 6	36 ± 6	35 ± 6	36 ± 6	36 ± 7	35 ± 6[a,b]
Small, μmol/L	19 ± 5	19 ± 5	19 ± 5	20 ± 5	20 ± 6	18 ± 6[a,b]
Medium, μmol/L	12 ± 5	12 ± 6	12 ± 5	12 ± 6	12 ± 6	12 ± 6
Large, μmol/L	3.9 ± 2.5	4.1 ± 2.5	4.1 ± 2.5	4.1 ± 2.5	4.6 ± 2.7[a]	5.1 ± 2.9[a,b]

Particle concentrations are presented for subgroups of each major lipid fraction. Size values present the average particle size across all lipoprotein subgroups. Baseline and year 1 data are presented as mean (SD).

[a] Treatment group comparisons at baseline resulted in $P < .01$ vs placebo.

[b] Treatment group comparisons at year 1 resulted in $P < .01$ vs metformin.

There were similar although more modest changes compared with placebo in the metformin group (all $P < .01$). The LDL size increased significantly in the ILS and metformin groups, with the ILS-associated increase greater than with metformin (Figure 1D; $P < .01$).

HDL size and large HDL-P increased in the ILS and metformin groups compared with placebo (Figure 1, C and D), whereas small HDL-P was reduced in the ILS but increased in the metformin group (all $P < .01$). These differences in HDL responses to metformin and ILS demonstrate the subfraction heterogeneity that may accompany changes in HDL-C. When the same analyses were conducted with adjustment for lipid-lowering medications, the same patterns were found (data not shown).

Lipoprotein subfractionation by DGU

Figure 2 depicts mean changes at 1 year for the three intervention groups. In the metformin and placebo groups, there was a slight reduction in buoyant VLDL, with a reciprocal increase in the denser fractions, whereas ILS reduced buoyant VLDL twice as much ($P < .001$ for overall VLDL change in ILS compared with placebo and metformin) as well as reducing the denser VLDL fractions. ILS reduced IDL levels ($P < .002$ vs metformin and $P < .001$ vs placebo) and led to a more robust decrease in dense and a reduction in buoyant LDL factions (overall LDL change in ILS compared with placebo, $P < .001$, and metformin, $P < .02$) compared with more modest decreases in the dense LDL fractions in the metformin ($P = .03$ vs placebo) and placebo groups in which buoyant LDL either did not change or increased slightly. HDL increased modestly and similarly in all three intervention groups. Overall the observations made with ultracentrifugation were parallel to those made using the NMR technique for VLDL and LDL, but ultracentrifugation does not discriminate as well for HDL subfractions.

Correlations between lipoproteins fractions by NMR and anthropometric and metabolic determinants at baseline

Table 3 displays Spearman correlations between study variables and lipoprotein P and size at baseline. HOMA-IR was directly correlated with large VLDL-P, VLDL size, and small LDL-P and inversely with large LDL-P and LDL size. BMI and waist circumference had similar although overall less robust associations with these measures, with the r values slightly greater for BMI than for waist circumference. Conversely, adiponectin levels correlated inversely with large VLDL-P, VLDL size, and small LDL-P and directly with large LDL-P and LDL size. HOMA-IR, BMI, and waist circumference were inversely and adiponectin directly correlated with large HDL-P and HDL size. Associations with small HDL-P were opposite to those with large HDL-P, although considerably weaker. Fasting and 2-hour glucose were directly correlated with small LDL-P, whereas fasting glucose was inversely related with LDL and HDL size and large HDL-P. Saturated fat intake correlated with VLDL size, and physical activity had no association with lipoprotein subfractions. Triglyceride level associated strongly with VLDL size and large

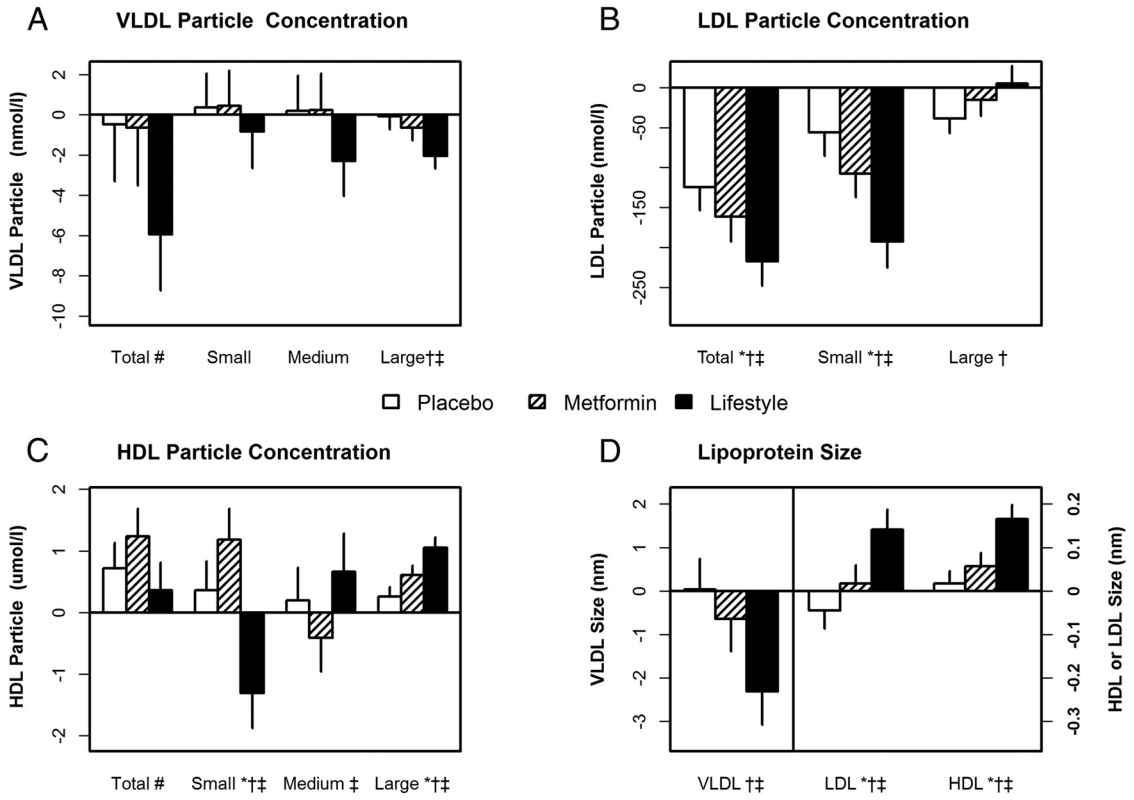

Figure 1. Changes from baseline in lipoprotein subfractions and size using NMR after 1 year according to treatment group. A, VLDL-P; B, LDL-P; C, HDL-P; D, Lipoprotein size. *, Adjusted $P < .01$ for placebo vs metformin; †, adjusted $P < .01$ for placebo vs lifestyle; ‡, adjusted $P < .01$ for metformin vs lifestyle; #, treatment group comparison not presented. Changes in subfractions were adjusted for baseline levels and use of lipid-lowering medications.

and medium VLDL P as well as with small LDL-P and correlated inversely with large LDL-P and large HDL-P. With a few exceptions, the associations of lipoprotein subfraction changes during the 1-year intervention period with changes in HOMA-IR, BMI, adiponectin, and glucose measures were similar to those seen in the baseline analysis, although these findings were more robust in the ILS than the other groups (data not shown).

Multivariate analysis of effect of determinants on change in lipoproteins

The effects of baseline and 1-year change in HOMA-IR, BMI, and adiponectin on adjusted ILS- and metformin-associated changes in selected lipoprotein subfractions at 1 year compared with placebo were examined in individual multivariate models as shown in the Web Appendix (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) and in a fully adjusted, combined model (Tables 4 and 5). BMI was chosen over waist circumference in these analyses because it correlated more strongly with subfraction change (BMI vs waist; VLDL size, r = 0.21* vs 0.14*; large VLDL-P: r = 0.20* vs 0.14*; LDL size: r = −0.19* vs −0.14*; small LDL-P: r = 0.23* vs 0.16*; large LDL-P: r = −0.09* vs −0.03; large HDL-P: r = −0.23* vs −0.19*; * $P < .01$). In individual models, the ILS-associated reduction in large VLDL-P diminished after adjustment for HOMA-IR change and was no longer significant after adjustment for BMI change; in the fully adjusted model, the large VLDL-P change was primarily associated with baseline and change in HOMA-IR (Table 4). BMI change had the largest influence on intervention-associated small LDL-P reduction in individual models and in the combined model, with a small residual unexplained ILS effect. All three metabolic variables contributed modestly in individual models to the increases in large LDL-P, but baseline and adiponectin change contributed mostly in the combined model with a residual effect of ILS unaccounted for. Similar observations were noted for LDL size change, except that BMI change remained significantly associated in the combined model (Table 5). In individual models, BMI and adiponectin changes were the major factors accounting for the intervention-associated increases in large HDL-P. This was true also for the ILS-associated small HDL-P decrease, but the metformin-associated increase in small HDL-P was unaccounted for. In the combined model, adiponectin change had the largest impact on the intervention effect. Baseline BMI and adiponectin and BMI change in the combined model accounted for

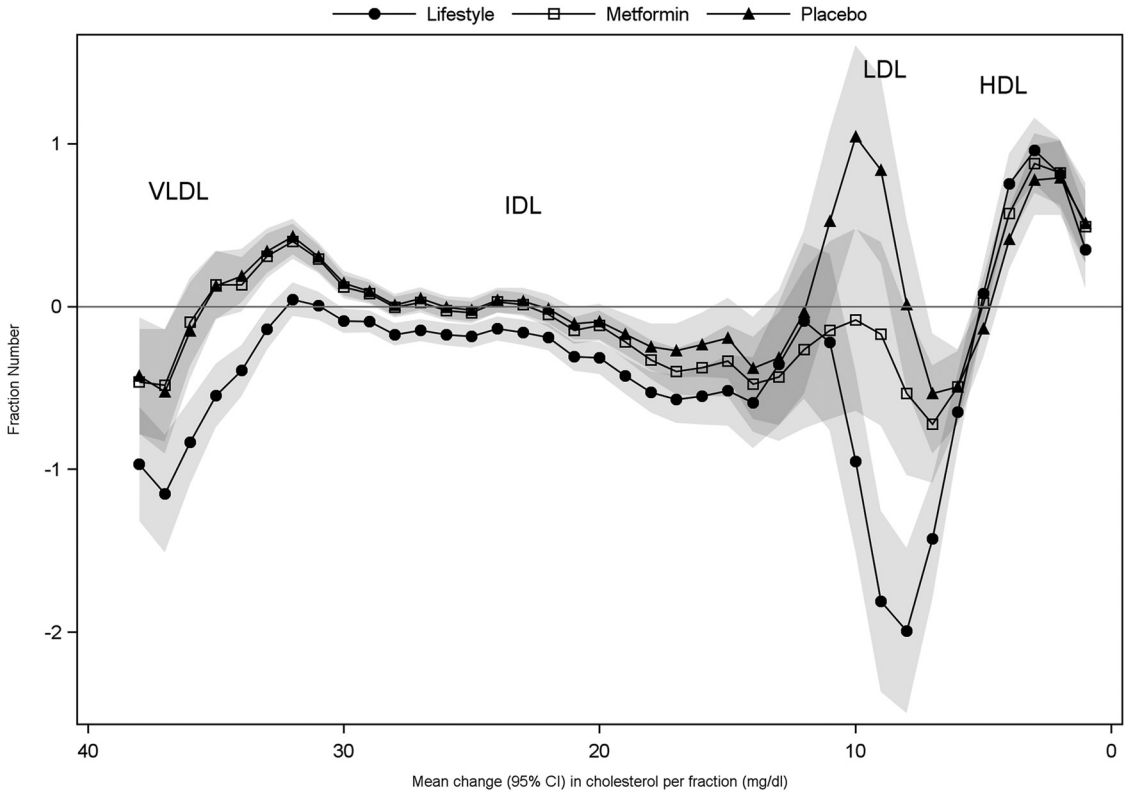

Figure 2. Mean changes [95% confidence interval (CI)] in lipoprotein subfraction distribution at 1 year according to treatment group using DGU. The approximate positions of VLDL, IDL, LDL, and HDL are shown. HDL is typically located in fractions 0–6, LDL in fractions 7–18, IDL in fractions 19–30, and VLDL in fractions 31–38.

much of the variance in small HDL-P change associated with ILS intervention but not with metformin. Both interventions had significant but opposite residual effects on small HDL-P.

Discussion

We used a combination of DGU to characterize the changes in lipoprotein density together with NMR to subfractionate lipoproteins by size and particle concentration in studying the effects of long-term lifestyle change or metformin treatment in a subgroup of the DPP cohort. These related yet differing methodologies have diverse strengths and weaknesses. Although the former has been well established, it is generally more expensive and time consuming than the latter and is less often used. Thus, the use of these two techniques in the same study provides an uncommon opportunity to corroborate the findings with the two methods and to contribute complementary size and density information on VLDL and LDL subfraction changes. In addition, NMR

Table 3. Adjusted Univariate Spearman Correlations of Baseline Lipoprotein Subfractions and Metabolic Characteristics[a]

Baseline Covariate	VLDL-P					LDL-P				HDL-P				
	Size	Total	Small	Medium	Large	Size	Total	Small	Large	Size	Total	Small	Medium	Large
HOMA IR	**0.35**[b]	**0.09**[c]	**−0.09**[b]	**0.13**[b]	**0.34**[b]	**−0.27**[b]	**0.08**[c]	**0.23**[b]	**−0.21**[b]	**−0.14**[b]	**−0.11**[b]	**0.10**[b]	**−0.12**[b]	**−0.25**[b]
BMI	**0.19**[b]	**−0.10**[b]	**−0.13**[b]	**−0.06**[c]	**0.11**[b]	**−0.11**[b]	**0.07**[c]	**0.13**[b]	**−0.08**[c]	**−0.15**[b]	**−0.12**[b]	**0.07**[c]	**−0.13**[b]	**−0.17**[b]
Waist	**0.20**[b]	**−0.09**[c]	**−0.12**[b]	**−0.06**	**0.13**[b]	**−0.12**[b]	**0.08**[c]	**0.14**[b]	**−0.08**[c]	**−0.17**[b]	**−0.12**[b]	**0.08**[c]	**−0.12**[b]	**−0.18**[b]
Physical activity (MAQ)	−0.05	0.03	0.02	0.04	−0.02	0.01	−0.01	−0.02	0.02	0.04	**0.06**[c]	0.02	0.01	0.04
Saturated Fat	**0.11**[b]	−0.03	−0.05	−0.02	**0.10**[b]	−0.04	0.00	0.03	−0.04	−0.05	−0.04	0.05	−0.06	−0.03
Fasting glucose	0.05	0.04	0.04	0.03	0.05	**−0.09**[c]	**0.07**[c]	**0.10**[b]	−0.03	**−0.12**[b]	**−0.12**[b]	−0.00	−0.05	**−0.15**[b]
2 h glucose	**0.12**[b]	0.01	−0.05	0.04	**0.11**[b]	**−0.08**[c]	0.05	**0.08**[c]	−0.06	0.01	0.03	0.01	0.02	−0.02
HbA1c	0.01	−0.02	0.03	−0.05	−0.00	0.02	**0.09**[c]	0.04	**0.06**[c]	**−0.13**[b]	**−0.08**[c]	−0.01	−0.03	**−0.10**[b]
Adiponectin	**−0.18**[b]	**−0.12**[b]	0.02	**−0.15**[b]	**−0.20**[b]	**0.27**[b]	**−0.11**[b]	**−0.24**[b]	**0.18**[b]	**0.17**[b]	**0.16**[b]	**−0.12**[b]	**0.15**[b]	**0.26**[b]
Triglyceride	**0.51**[b]	**0.53**[b]	0.06	**0.58**[b]	**0.68**[b]	**−0.55**[b]	**0.29**[b]	**0.50**[b]	**−0.41**[b]	**−0.06**[c]	0.06	**0.12**[b]	0.04	**−0.27**[b]

[a] All Spearman correlations are adjusted for age at randomization, sex, and race/ethnicity and in bold if $P < .01$.

[b] $P < .0001$.

[c] $P < .01$.

Table 4. Fully Adjusted Effects of ILS, Metformin, and Metabolic Parameters on Changes in Selected VLDL and LDL Subfractions After 1 Year of Intervention[a]

	Δ Large VLDL-P		Δ Small LDL-P		Δ Large LDL-P	
Effect	**β (95% CI)**	**R^2**	**β (95% CI)**	**R^2**	**β (95% CI)**	**R^2**
Demographic adjusted model		22%		22%		18%
ILS vs placebo	**−1.9 (−2.6, −1.2)**	**1.9%**	**−133 (−166, −101)**	**3.9%**	**47 (24, 69)**	**1%**
MET vs placebo	−0.53 (−1.2, 0.14)	0.1%	**−52 (−83, −20)**	**0.6%**	**26 (4.1, 48)**	**0.3%**
Fully adjusted model†		28%		29%		20%
ILS vs placebo	−0.54 (−1.3, 0.22)	0.1%	**−50 (−85, −15)**	**0.5%**	**27 (1.6, 53)**	**0.3%**
MET vs placebo	0.22 (−0.47, 0.91)	0%	−14 (−45, 18)	0%	14 (−9.1, 37)	0.1%
Bas log HOMA-IR	**1.0 (0.65, 1.4)**	**1.7%**	**23 (5.8, 41)**	**0.4%**	**−15 (−28, −2.0)**	**0.3%**
Δ log HOMA-IR (1 SD)	**1.3 (0.91, 1.6)**	**3%**	**28 (11, 45)**	**0.7%**	**−16 (−29, −4.3)**	**0.5%**
Baseline BMI (1 SD)	−0.18 (−0.53, 0.16)	0.1%	10 (-5.8, 26)	0.1%	2.2 (−9.5, 14)	0%
Δ BMI (1 SD)	**0.69 (0.33, 1.0)**	**0.9%**	**53 (37, 70)**	**2.5%**	−0.09 (−12, 12)	0%
Baseline APN (1 SD)	−0.11 (−0.45, 0.23)	0%	**−35 (−51, −19)**	**1.2%**	**25 (13, 36)**	**1.2%**
Δ APN (1 SD)	0 (−0.30, 0.30)	0%	**−32 (−46, −18)**	**1.3%**	**19 (9.1, 29)**	**0.9%**

Abbreviation: APN, adiponectin; CI, confidence interval.

[a] The effects were estimated in regression models in which baseline and changes in metabolic parameters (BMI, log HOMA-IR, and APN) were standardized. Significant treatment and covariate effects are highlighted in bold.

† All models had the 1-year change in lipid subfraction as dependent variable with adjustments for the baseline lipoprotein subfraction or particle size and demographic covariates (age at randomization, sex, and race/ethnicity) in the first model and baseline and 1-year changes in adiponectin + BMI + log HOMA-IR in the fully adjusted model.

is able to generate detailed HDL subfraction analysis of size and particle concentration that was not possible using the DGU procedure.

Using these two lipoprotein subfractionation technologies, we show that previously documented changes in triglyceride and HDL-C but not LDL-C (11) are accompanied by apparently favorable and widespread changes in VLDL, IDL, LDL, and HDL particle concentration, size, and density. ILS had significant effects on all lipoprotein classes. First, the intervention reduced the concentration of large and medium VLDL-P, thus decreasing VLDL size, corresponding with the lowering of both buoyant and denser VLDL subfractions. Second, ILS decreased small LDL-P and ultracentrifugally dense LDL and modestly increased large LDL-P by NMR, leading to an overall enlargement of LDL but reduction in total LDL P. Third,

Table 5. Fully Adjusted Effects of ILS, Metformin, and Metabolic Parameters on Changes in Selected Lipoprotein HDL Subfractions and LDL Size After 1 Year of Intervention[a]

	Δ Small HDL-P		Δ Large HDL-P		Δ LDL size	
Effect	**β (95% CI)**	**R^2**	**β (95% CI)**	**R^2**	**β (95% CI)**	**R^2**
Demographic adjusted model		18%		9.5%		17%
ILS vs placebo	**−1.7 (−2.2, −1.1)**	**2.1%**	**0.78 (0.59, 0.96)**	**4%**	**0.19 (0.14, 0.24)**	**3.4%**
MET vs placebo	**0.76 (0.22, 1.3)**	**0.5%**	**0.36 (0.18, 0.54)**	**0.9%**	**0.06 (0.02, 0.11)**	**0.4%**
Fully adjusted model†		21%		20%		26%
ILS vs placebo	**−1.0 (−1.7, −0.40)**	**0.7%**	**0.32 (0.12, 0.52)**	**0.6%**	**0.07 (0.02, 0.13)**	**0.5%**
MET vs placebo	**1.1 (0.52, 1.7)**	**0.9%**	**0.21 (0.03, 0.39)**	**0.3%**	0.01 (−0.036, 0.06)	0%
Bas log HOMA-IR	−0.12 (−0.43, 0.19)	0%	**−0.17 (−0.27, −0.07)**	**0.8%**	**−0.04 (−0.06, −0.01)**	**0.5%**
Δ log HOMA-IR (1 SD)	0.01 (−0.29, 0.30)	0%	**−0.10 (−0.19, 0.00)**	**0.3%**	**−0.06 (−0.08, −0.03)**	**1.2%**
Baseline BMI (1 SD)	**0.35 (0.06, 0.63)**	**0.4%**	0.00 (−0.09, 0.09)	0%	−0.003 (−0.03, 0.02)	0%
Δ BMI (1 SD)	**0.59 (0.30, 0.89)**	**1%**	**−0.23 (−0.32, −0.13)**	**1.4%**	**−0.05 (−0.08, −0.03)**	**1.1%**
Baseline APN (1 SD)	**−0.67 (−0.95, −0.38)**	**1.4%**	**0.18 (0.09, 0.28)**	**1%**	**0.08 (0.06, 0.11)**	**3%**
Δ APN (1 SD)	−0.069 (−0.32, 0.18)	0%	**0.34 (0.26, 0.42)**	**4.4%**	**0.07 (0.04, 0.09)**	**2.4%**

Abbreviation: APN, adiponectin; CI, confidence interval.

[a] The effects were estimated in regression models in which baseline and changes in metabolic parameters (BMI, log HOMA-IR, and APN) were standardized. Significant treatment and covariate effects are highlighted in bold.

† All models had the 1-year change in lipid subfraction as dependent variable with adjustments for the baseline lipoprotein subfraction or particle size and demographic covariates (age at randomization, sex, and race/ethnicity) in the first model and baseline and 1-year changes in adiponectin + BMI + log HOMA-IR in the fully adjusted model.

there was a 24% rise in large HDL-P concentrations, resulting in increased HDL size. These changes are all considered to reduce the atherogenicity of the lipoprotein profile. Although the mechanisms for these effects are not completely understood, overproduction of large, buoyant VLDL-P is believed to be a primary abnormality in insulin-resistant states (12), leading to the remodeling of LDL and HDL to proatherogenic smaller, denser particles (13, 14). Weight reduction reverses these abnormalities (15, 16). The decrease in small dense LDL likely reflects reduced triglyceride-cholesterol ester exchange, favoring formation of larger buoyant LDL, which may be less atherogenic (17). These changes were accompanied by a reduction in total LDL-P concentration, which could result from the more rapid clearance of larger LDL-P (18). This is clinically relevant because total LDL-P has been shown to correlate more strongly with cardiovascular disease occurrence than LDL-C (19), which did not change in the DPP (2). An increase in the proportion of large to small HDL-P, likely due in part to reduced particle remodeling, has also been associated with reduced cardiovascular disease risk (20). Overall, the ILS-associated reductions in VLDL and small dense LDL and the increase in the proportion of large to small HDL-P are considered favorable changes.

Metformin had no effect on VLDL but was associated with LDL subfraction changes similar to those seen with ILS, although of smaller magnitude. Not previously described was the observation that metformin increased both large and small HDL-P. Unlike ILS, these LDL and HDL changes were not accompanied by changes in triglyceride or VLDL-P concentrations, suggesting a mechanism independent of triglyceride/VLDL change. The clinical significance of the increase in small HDL-P is unclear (20). A known example of a pharmacological agent increasing small HDL-P is gemfibrozil treatment, and in the Veteran Administration HDL Intervention Trial, the gemfibrozil-induced increase in small HDL-P was associated with a reduction in cardiovascular events (21). Although the basis for these metformin-induced changes in HDL is unknown, it is clear that metformin and ILS alter HDL in different ways.

What were the possible determinants of these lipoprotein changes? Reduction in saturated fat and increase in physical activity, key elements of the ILS intervention, may alter lipoprotein subfractions (22, 23). However, these factors had little effect on the noted changes. Modest improvements in dysglycemia occurred with both interventions, but the contribution of these effects was relatively small. More important appeared to be the change in BMI or waist circumference. Although waist circumference and BMI had similar associations with lipoprotein subfractions and correlated very strongly ($r = 0.90$), change in BMI was preferred in this analysis because it was more strongly correlated with lipoprotein change than waist circumference in this very obese population. BMI correlated positively with large VLDL-P, VLDL size, and small LDL-P concentrations but inversely with large HDL-P. Because the lipoprotein changes in the ILS group were all associated with BMI, it is very likely that that the weight reduction achieved by the lifestyle change led to most of these effects. The mechanisms linking BMI change and lipoprotein modification are complex because weight change results in several metabolic alterations impacting lipoprotein metabolism among which increased insulin sensitivity is clearly important. In this regard there is interest in the role of the insulin-sensitizing cytokine adiponectin on lipoproteins because adiponectin levels are known to associate strongly with HDL-C and inversely with triglyceride concentrations (24). We therefore examined the separate and combined effects of these factors on selected lipoprotein subfraction measures in multivariate models. Because of the possibility that there were weight-independent effects of metformin on lipoproteins, both treatment effects compared with placebo were included as separate model variables.

BMI and HOMA-IR accounted for nearly all of the ILS effect on large VLDL-P concentrations, with the change in HOMA-IR being the most important factor. This is in accord with evidence that insulin resistance enhances VLDL secretion and increased large VLDL-P production (12). HOMA-IR had a smaller influence on the ILS and metformin effects on LDL subfractions, with both BMI and adiponectin accounting for most of the change in small LDL-P, whereas adiponectin was most strongly associated with the changes in large LDL-P concentration and LDL size. There are reports that weight loss is accompanied by a reduction in cholesteryl ester transfer protein as well as hepatic lipase activity (16, 25) The latter may be due to an increase in adiponectin levels (26), which we have previously shown occurred with the ILS intervention (27). These changes would be expected to reduce small LDL-P formation, and decreased hepatic lipase could explain the association between adiponectin and large LDL-P changes (28). Adiponectin has also been shown to increase lipoprotein lipase (29), which together with hepatic lipase are important regulators of HDL metabolism (30). Because reduced hepatic lipase and increased lipoprotein lipase activities would be expected to increase HDL size, this could explain why adiponectin change was a stronger determinant of ILS treatment effects on large HDL-P than BMI or HOMA-IR. All three variables contributed to the ILS-associated small HDL-P decrease but had no effect on the increase in this subclass in metformin-treated subjects. These findings support the thesis that

most of the ILS-associated HDL-P and size changes reflect a remodeling of small to large HDL-P, whereas metformin also appears to independently increase small HDL-P. These observations raise the possibility that intervention-associated increases in adiponectin levels play a significant role in the subsequent effects on lipoprotein subfractions.

In summary, we show for the first time in a year-long study involving a large, multiethnic high-risk group that ILS and metformin treatments, previously demonstrated to slow diabetes development, also produced widespread and mostly favorable alterations in lipoprotein subfractions, using techniques that yielded corroborative changes in size and density. These effects were seen after a year of the study interventions, raising the possibility for sustained benefits beginning soon after initiation of treatment. We demonstrate that the ILS effect on lipoproteins appears to be largely explained by the accompanying weight reduction and associated metabolic changes in insulin resistance and adiponectin levels and therefore appears to parallel the actions of ILS in slowing diabetes development (31). In contrast, the effect of metformin appears to involve both weight reduction and other specific but unknown treatment influences (32), particularly on HDL. Overall, the findings offer optimism that these interventions in addition to slowing diabetes development may also slow the progression of atherosclerosis.

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A complete list of centers, investigators, and staff can be found in the Appendix.

This study is registered at Clinical Trials, number NCT00004992.

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A complete list of investigators can be found in the Appendix.

Disclosure Summary: M.T., J.B., S.M.M., K.J.M., K.E.W., R.F.A., E.S.H., and E.B.-C. having nothing to declare. R.B.G. has consulted for Merck, Daiichi, and LipoScience and served on an Advisory Board for Bristol Myers Squibb/Astra Zeneca, and J.O. is employed by and owns stock in LipoScience, Inc.

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