



# HDL-C and HDL-C/ApoA-I Predict Long-Term Progression of Glycemia in Established Type 2 Diabetes

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## OBJECTIVE

Low HDL cholesterol (HDL-C) and small HDL particle size may directly promote hyperglycemia. We evaluated associations of HDL-C, apolipoprotein A-I (apoA-I), and HDL-C/apoA-I with insulin secretion, insulin resistance, HbA<sub>1c</sub>, and long-term glycemic deterioration, reflected by initiation of pharmacologic glucose control.

## RESEARCH DESIGN AND METHODS

The 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study followed 9,795 type 2 diabetic subjects. We calculated baseline associations of fasting HDL-C, apoA-I, and HDL-C/apoA-I with HbA<sub>1c</sub> and, in those not taking exogenous insulin ( $n = 8,271$ ), with estimated  $\beta$ -cell function (homeostasis model assessment of  $\beta$ -cell function [HOMA-B]) and insulin resistance (HOMA-IR). Among the 2,608 subjects prescribed lifestyle only, Cox proportional hazards analysis evaluated associations of HDL-C, apoA-I, and HDL-C/apoA-I with subsequent initiation of oral hypoglycemic agents (OHAs) or insulin.

## RESULTS

Adjusted for age and sex, baseline HDL-C, apoA-I, and HDL-C/apoA-I were inversely associated with HOMA-IR ( $r = -0.233$ ,  $-0.134$ , and  $-0.230$ ; all  $P < 0.001$ ;  $n = 8,271$ ) but not related to HbA<sub>1c</sub> (all  $P > 0.05$ ;  $n = 9,795$ ). ApoA-I was also inversely associated with HOMA-B ( $r = -0.063$ ;  $P = 0.002$ ;  $n = 8,271$ ) adjusted for age, sex, and HOMA-IR. Prospectively, lower baseline HDL-C and HDL-C/apoA-I levels predicted greater uptake (per 1-SD lower: hazard ratio [HR] 1.13 [CI 1.07–1.19],  $P < 0.001$ ; and HR 1.16 [CI 1.10–1.23],  $P < 0.001$ , respectively) and earlier uptake (median 12.9 and 24.0 months, respectively, for quartile 1 vs. quartile 4; both  $P < 0.01$ ) of OHAs and insulin, with no difference in HbA<sub>1c</sub> thresholds for initiation ( $P = 0.87$  and  $P = 0.81$ ). Controlling for HOMA-IR and triglycerides lessened both associations, but HDL-C/apoA-I remained significant.

## CONCLUSIONS

HDL-C, apoA-I, and HDL-C/apoA-I were associated with concurrent insulin resistance but not HbA<sub>1c</sub>. However, lower HDL-C and HDL-C/apoA-I predicted greater and earlier need for pharmacologic glucose control.

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Type 2 diabetes is a progressive disease, characterized by insulin resistance and ongoing loss of endogenous insulin secretion with increased requirement for pharmacologic glucose control over time (1,2). Low HDL cholesterol (HDL-C) is a common finding in type 2 diabetic patients and best known as a predictor of cardiovascular risk (3–5). Furthermore, changes to HDL-C levels, HDL particles, and their major apolipoprotein, apolipoprotein A-I (apoA-I), are reported to be present years before the development of type 2 diabetes (6–12). This raises the question of whether HDL biology contributes directly to the development of type 2 diabetes and the continuing progression of the disease.

Associations between HDL-related measures and incident type 2 diabetes may reflect comorbid conditions such as hypertriglyceridemia, abdominal obesity, and insulin resistance, which also portend incident disease. However, recent preclinical evidence suggests that the action of HDL particles and apoA-I can independently promote insulin secretion and glucose uptake in patients with type 2 diabetes (13,14). Human islet-cell culture and animal studies have reported that exogenous HDL improves insulin secretion through increased reverse cholesterol transport and attenuation of LDL and inflammation-induced apoptosis of pancreatic  $\beta$ -cells (13,15). HDL and apoA-I may also promote glucose uptake by skeletal muscle through activating the AMPK pathway (16). In humans with type 2 diabetes, measures of endogenous HDL function have been inversely associated with concurrent estimated  $\beta$ -cell function (17). Also, infusion of exogenous reconstituted HDL over 4 h increased both insulin secretion and skeletal muscle glucose uptake in a small trial with type 2 diabetic patients (18). Cross-sectional studies have reported an inverse association between HbA<sub>1c</sub> and HDL-C in type 2 diabetes (19,20). Further, a number of studies have linked HDL-C levels to the development of incident type 2 diabetes (6–8,10). However, to date, no study has examined the relationship between these biomarkers and the progression of established diabetes.

The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial was a randomized, controlled trial of fenofibrate therapy in 9,795 adult type

2 diabetic patients with measures of HDL-C and apoA-I at baseline. We investigated whether HDL-C, apoA-I, and HDL particle size, as estimated by the HDL-C/apoA-I ratio (21), were inversely associated with cross-sectional measures of glycemia and prospectively with requirements for escalation of glucose control therapies, which would be consistent with HDL having antidiabetic effects. We also considered associations of the HDL-related measures with two key determinants of glycemia, pancreatic  $\beta$ -cell secretion and insulin resistance.

## RESEARCH DESIGN AND METHODS

### Subjects

The FIELD study design has been published previously (22,23). All participants had an initial total plasma cholesterol of 3.0–6.5 mmol/L, plus a total cholesterol to HDL-C ratio of  $\geq 4.0$  or a fasting plasma triglyceride reading of 1.0–5.0 mmol/L. The study excluded those who had an existing indication for lipid-modifying therapy, renal impairment (plasma creatinine  $\geq 130$   $\mu$ mol/L), chronic liver disease (alanine aminotransferase [ALT]  $\geq 2$  times the upper limit of normal), symptomatic gallbladder disease, or those who had experienced a cardiovascular event within the 3 months before recruitment. Patients were followed up every 4 months in the first year and then every 6 months for a median of 5 years.

### Measurements and Outcomes

Fasting serum HDL-C, serum apoA-I, plasma glucose, serum insulin, and HbA<sub>1c</sub> were measured at baseline in two core laboratories aligned to the Canadian Reference Laboratory. We then calculated the HDL-C/apoA-I ratio. In subjects not taking exogenous insulin ( $n = 8,271$ ), homeostasis model assessment (HOMA; v2.2.2, <http://www.dtu.ox.ac.uk>) was used to derive estimates of pancreatic  $\beta$ -cell secretion (HOMA-B) and insulin resistance (HOMA-IR) from fasting plasma glucose and serum insulin (24). As recommended by the model's authors, HOMA calculations were further limited to patients with glucose readings between 3 and 25 mmol and insulin levels between 20 and 400 pmol (25). Medications were recorded at each visit. Baseline adiposity was assessed using BMI, waist circumference, and waist-to-hip ratio. Renal function

was assessed using both estimated glomerular filtration rate (eGFR) and the presence of albuminuria. Evidence of fatty liver change was inferred from ALT readings. Self-reported smoking status, regular alcohol consumption, and physical exercise were recorded at baseline. At all centers, medication use was recorded at baseline and at each subsequent visit changes were recorded by a qualified clinical trials nurse using a common drug dictionary, which was later converted to Anatomical Therapeutic Chemical Classification System code. For patients commencing the trial on lifestyle measures alone, we regarded initiation of oral hypoglycemic agents (OHAs) or insulin therapy by the patients' usual general practitioner or endocrinologist, once confirmed, as evidence of escalation of diabetes therapy.

### Statistical Analysis

We first considered age and sex-adjusted partial correlations between baseline variables, which were logarithmically transformed as appropriate. Partial correlations with HOMA-B were additionally adjusted for HOMA-IR. Linear regression analysis was used in order to further adjust for possible confounders, which were selected based on known or suspected association with the variables of interest. All baseline analyses were repeated in the lifestyle-only subgroup ( $n = 2,608$ ) to avoid possible confounding effects of OHAs or insulin therapy. In the same subgroup, we used Cox proportional hazards models to consider whether lower HDL-C, apoA-I, or HDL-C/apoA-I was associated with more rapid uptake of OHAs and insulin therapy during follow-up. Interaction terms for treatment allocation were evaluated. Models were adjusted for HOMA-IR, BMI, HbA<sub>1c</sub>, triglycerides, waist circumference, hypertension, LDL-C, ALT, eGFR, the presence of albuminuria, current smoking, regular alcohol consumption, physical exercise, and menopause status in women. In separate time-dependent Cox regression analyses, we evaluated the effect of uptake of statins and ACE inhibitors (ACEi)/angiotensin receptor blockers (ARBs) during follow-up. Where an HDL-related measure was found to be a significant predictor of progression to pharmacologic glucose control, we aimed to exclude possible interactions

between the measure and glycemic management. We classed participants into sex-stratified quartiles according to each relevant measure. Using a one-way ANOVA, we assessed whether the highest quartile of the measure was associated with a greater rise in HbA<sub>1c</sub> than the lowest quartile. In those who initiated pharmacologic glucose control, we compared the last recorded HbA<sub>1c</sub> before therapeutic progression between the highest and lowest quartiles of a measure using a Kruskal-Wallis test. *P* values are presented unadjusted for multiple comparisons. All analyses used SPSS 20.0 (IBM, Armonk, NY) or SAS 9.1 software (SAS Institute, Cary, NC).

## RESULTS

The 9,795 FIELD participants had a mean age of 62 ± 7 years with a median diabetes duration of 5 years (interquartile range [IQR] 2–10 years), median HbA<sub>1c</sub> of 6.8% (IQR 6.1–7.8%) (51 mmol/mol [IQR 43–62 mmol/mol]), and mean HDL-C of 1.10 (SD 0.27)

mmol/L. We recorded Caucasian ethnicity for 93% of participants. The 2,608 participants in the lifestyle-only subgroup tended to have a shorter duration of diabetes (2.0 vs. 5.0 years), a lower HbA<sub>1c</sub> (6.0 vs. 6.8% [42 vs. 51 mmol/mol]) and a higher HOMA-B score (64.2 vs. 50.2) than other participants in the FIELD cohort but were otherwise comparable. HOMA-B and HOMA-IR values were calculated for 8,271 participants in the general cohort and 2,560 participants in the lifestyle-only subgroup. Baseline characteristics for the entire cohort as well as for subgroups on hypoglycemic pharmacotherapy and on lifestyle measures only are presented in Table 1.

Baseline age- and sex-adjusted partial correlations are shown in Table 2. There were no significant partial correlations of HDL-C, apoA-I, or HDL-C/apoA-I with HbA<sub>1c</sub> in the whole cohort (*n* = 9,795) or the lifestyle-only subset (*n* = 2,608). This persisted after additional adjustment, by linear regression, for possible confounders, which were

duration of diabetes, adiposity, triglycerides, HOMA-IR, renal function, and ALT. HOMA-IR was inversely correlated to HDL-C, apoA-I, and HDL-C/apoA-I (*r* = −0.223, −0.134, and −0.230, respectively, in the whole cohort [*N* = 8,271]; all *P* < 0.001), which persisted following further adjustment for possible confounders. Adjusted for age, sex, and HOMA-IR, there was a small inverse partial correlation between HOMA-B and apoA-I in both the whole cohort and lifestyle-only cohort (*r* = −0.041, *P* < 0.001; and *r* = −0.063, *P* = 0.002, respectively) which persisted following adjustment for BMI, waist-to-hip ratio, triglyceride levels, inferred renal function, ALT levels, and diabetes duration.

Over a median follow-up period of 5 years, 1,520 (58.3%) of the 2,608 participants using lifestyle-only measures at baseline commenced an OHA or insulin therapy. Cox proportional hazards analysis, adjusted for age and sex, found that lower HDL-C and HDL-C/apoA-I, but not apoA-I, predicted a

**Table 1—Baseline characteristics for participants**

Characteristics	Lifestyle measures only ( <i>N</i> = 2,608)	OHAs and insulin therapy ( <i>N</i> = 7,187)	Entire cohort ( <i>N</i> = 9,795)	<i>P</i> value*
Male (%)	1,616 (62.0%)	4,522 (62.9%)	6,138 (62.7%)	0.387
Age (years)	62.2 (6.8)	62.2 (6.9)	62.2 (6.9)	0.622
Diabetes duration (years)	2.0 (1–5)	6 (3–11)	5.0 (2–10)	<0.001
BMI (kg/m <sup>2</sup> )	29.3 (26.4–33.0)	30.0 (27.0–33.8)	29.8 (26.7–33.5)	<0.001
Waist circumference (cm)	102 (13)	104 (13)	104 (13)	<0.001
Waist-to-hip ratio	0.92 (0.08)	0.94 (0.08)	0.93 (0.08)	<0.001
Laboratory data				
HbA <sub>1c</sub> (%)	6.0 (5.6–6.7)	7.2 (6.4–8.2)	6.8 (6.1–7.8)	<0.001
HbA <sub>1c</sub> (mmol/mol)	42 (38–50)	55 (46–66)	51 (43–62)	<0.001
Plasma glucose (mmol/L)	7.1 (6.2–8.2)	8.9 (7.4–10.9)	8.3 (6.9–10.3)	<0.001
Serum insulin (U/mL)	12 (8.0–17)	12 (8.0–19)	12 (8.0–18.5)	<0.001
LDL-C (mmol/L)	3.16 (0.67)	3.08 (0.66)	3.10 (0.66)	<0.001
HDL-C (mmol/L)	1.11 (0.27)	1.09 (0.26)	1.10 (0.27)	0.009
ApoA-I (g/L)	1.30 (0.21)	1.29 (0.21)	1.29 (0.21)	0.028
HDL-C/apoA-I (mg/mg)	0.33 (0.04)	0.32 (0.04)	0.32 (0.04)	0.020
Triglycerides (mmol/L)	1.68 (1.28–2.21)	1.74 (1.33–2.32)	1.72 (1.32–2.29)	<0.001
ALT (U/L)	22.5 (17.5–30.5)	24.0 (18.0–33.0)	23.5 (17.5–32.5)	<0.001
eGFR	88.2 (18.6)	88.0 (19.4)	88.1 (19.2)	0.703
Microalbuminuria†	555 (21.3%)	1,549 (21.6%)	2,104 (21.5%)	0.788
Macroalbuminuria†	97 (3.7%)	307 (4.3%)	404 (4.1%)	0.228
HOMA-derived values‡				
	<i>N</i> = 2,560	<i>N</i> = 5,711	<i>N</i> = 8,271	
HOMA-IR (ratio)	1.64 (1.09–2.44)	1.70 (1.15–2.52)	1.68 (1.12–2.48)	<0.001
HOMA-B (%)	64.2 (43.8–91.3)	43.6 (27.9–67.8)	50.2 (31.6–76.0)	<0.001

Continuous data are expressed as median (IQR) or mean (SD), and categorical data are shown as indicated. \*Statistical tests compared those commencing on lifestyle only measures to those commencing on OHAs and/or insulin therapy. *P* values were obtained using  $\chi^2$  tests for categorical variables, one-way ANOVA for normally distributed continuous variables, or Kruskal-Wallis tests for nonnormally distributed continuous variables. †Microalbuminuria is defined as urine albumin-to-creatinine ratio  $\geq 2.5$  and <25 mg/mmol for men and  $\geq 3.5$  and <35 mg/mmol for women. Macroalbuminuria is defined as urine albumin-to-creatinine ratio >25 mg/mmol for men and >35 mg/mmol for women. ‡The HOMA model defines HOMA-B of 100% and HOMA-IR of 1 as normal.

**Table 2—Age- and sex-adjusted associations of baseline HDL-related and glycemic variables (partial correlations)**

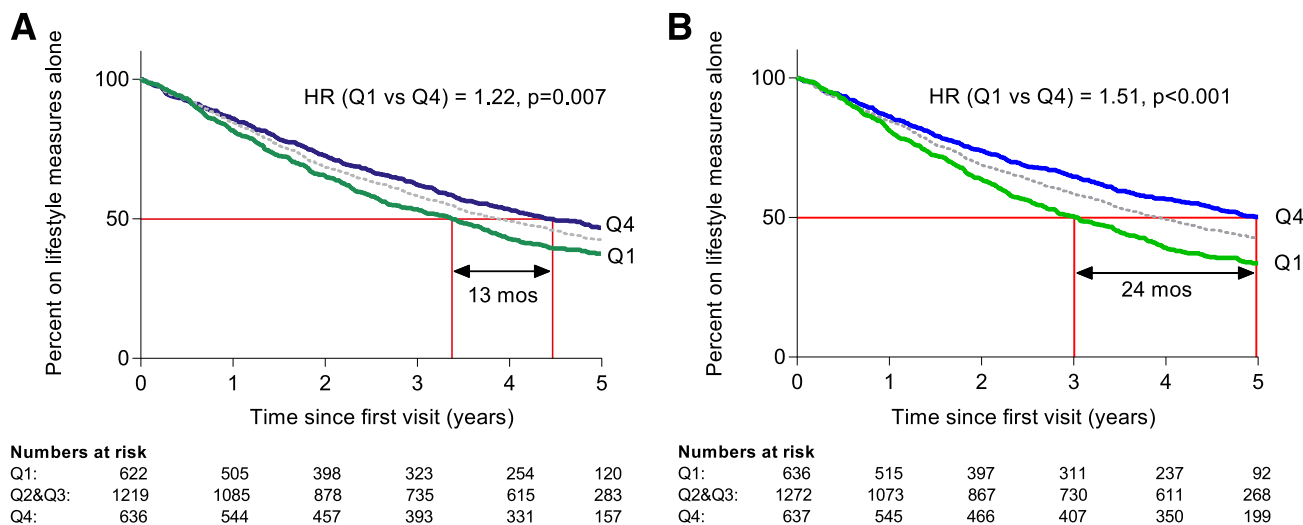
	HbA <sub>1c</sub>	HOMA-IR	HOMA-B	HOMA-B (adjusted for HOMA-IR)*
Whole cohort	N = 9,795		N = 8,271	
HDL-C	−0.009	−0.223 <sup>B</sup>	−0.128 <sup>B</sup>	−0.016
ApoA-I	0.002	−0.134 <sup>B</sup>	−0.103 <sup>B</sup>	−0.041 <sup>B</sup>
HDL-C/apoA-I	−0.001	−0.230 <sup>B</sup>	−0.100 <sup>B</sup>	0.021
Lifestyle only	N = 2,608		N = 2,560	
HDL-C	−0.029	−0.245 <sup>B</sup>	−0.174 <sup>B</sup>	−0.037
ApoA-I	−0.012	−0.169 <sup>B</sup>	−0.151 <sup>B</sup>	−0.063 <sup>A</sup>
HDL-C/apoA-I	−0.018	−0.254 <sup>B</sup>	−0.131 <sup>B</sup>	0.026

HbA<sub>1c</sub>, HOMA-IR, and HOMA-B were logarithmically transformed prior to correlation and regression analyses as described in the RESEARCH DESIGN AND METHODS section. <sup>A</sup>Correlation is significant at the 0.01 level (two-tailed). <sup>B</sup>Correlation is significant at the 0.001 level (two-tailed). \*Partial correlation adjusted for age, sex, and HOMA-IR.

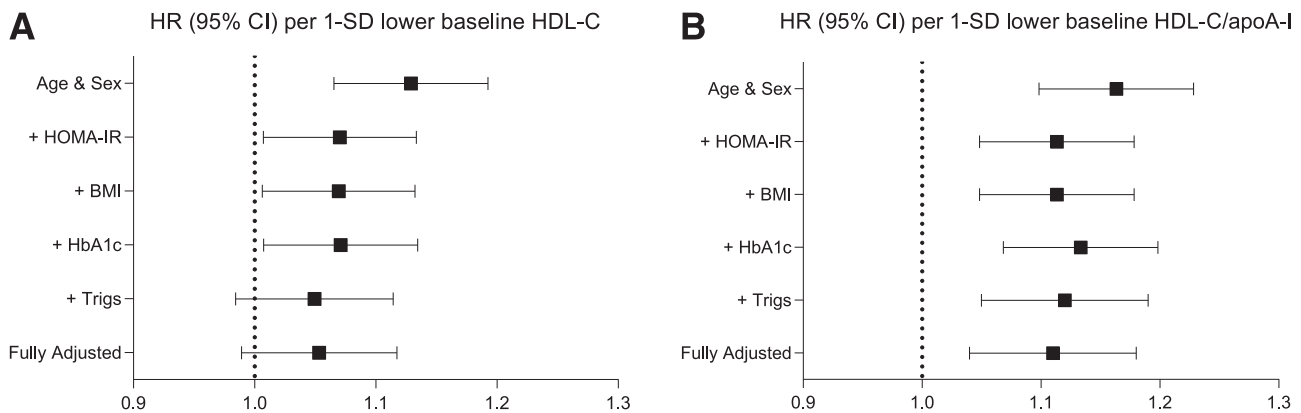
greater risk of initiation of OHAs or insulin therapy (HDL-C: hazard ratio [HR] 1.13 [CI 1.07–1.19], *P* < 0.001; apoA-I: HR 1.04 [CI 0.99–1.10], *P* = 0.102; and HDL-C/apoA-I: HR 1.16 [CI 1.10–1.23], *P* < 0.001, per 1-SD lower). As fenofibrate is known to affect HDL metabolism (26), we checked if there was any interaction between the HDL-related variables and treatment allocation. HDL-C and HDL-C/apoA-I were significant predictors of initiation of pharmacologic glucose control in both the fenofibrate and placebo arms of the study separately (HDL-C: HR 1.13, *P* = 0.003 [placebo arm] vs. HR 1.12, *P* = 0.003 [fenofibrate arm]; HDL-C/apoA-I: HR 1.13, *P* = 0.002 [placebo arm] vs.

HR 1.21, *P* < 0.001 [fenofibrate arm]), and the interaction terms with treatment allocation were nonsignificant for both HDL-C (*P* = 0.95) and HDL-C/apoA-I (*P* = 0.19). Those in the lowest baseline sex-stratified HDL-C quartile (median 0.83 mmol/L) progressed to pharmacologic glucose control a median of 13 months earlier than those in the highest quartile (median 1.40 mmol/L) (Fig. 1). Those in the lowest baseline sex-stratified HDL-C/apoA-I quartile (median 0.28) progressed to pharmacologic glucose control a median of 24 months earlier than those in the highest baseline quartile (median 0.37). Annualized rises in HbA<sub>1c</sub> did not differ significantly according to

baseline HDL-C quartile (0.079%/year [0.86 mmol/mol/year] quartile [Q1 vs. 0.073%/year [0.80 mmol/mol/year] Q4; *P* = 0.58) or baseline HDL-C/apoA-I quartile (0.076%/year [0.83 mmol/mol/year] Q1 vs. 0.057%/year [0.62 mmol/mol/year] Q4; *P* = 0.10). In those who progressed to pharmacologic glucose control, the median HbA<sub>1c</sub> values prior to initiation of pharmacologic glucose control did not differ significantly either for baseline HDL-C quartile (7.0% [53 mmol/mol] Q1 vs. 7.1% [54 mmol/L] Q4; *P* = 0.87) or HDL-C/apoA-I quartile (7.0% [53 mmol/mol] Q1 vs. 7.1% [54 mmol/L] Q4; *P* = 0.81). We then adjusted cumulatively for HOMA-IR, BMI, and HbA<sub>1c</sub>, which we had previously reported as determinants of progression to pharmacologic glucose control in part of the FIELD cohort (23) (Fig. 2). After further adjusting for triglycerides, only HDL-C/apoA-I remained a significant predictor of progression to pharmacologic glucose control over 5 years. Additional adjustment for waist-to-hip ratio, LDL-C, ALT, eGFR, albuminuria, current smoking, regular alcohol consumption, physical exercise, and menopause status in women did not significantly affect either predictor. Neither statin nor ACEi/ARB initiation during follow-up appreciably altered the prospective results (not shown). Similarly, inclusion of data on patients whose HOMA parameters are not recommended to be calculated



**Figure 1**—Kaplan-Meier curves showing percent of subjects remaining on lifestyle measures alone over time, according to baseline HDL-C (A) and HDL-C/apoA-I (B) quartiles (sex stratified). Q2 and Q3 are combined for clarity. The median values for Q1 and Q4 were 0.83 and 1.40 mmol/L for HDL-C and 0.28 and 0.37 mg/mg for HDL-C/apoA-I, respectively. The median times to progression to pharmacotherapy were 13 and 24 months earlier for Q1 than Q4 by HDL-C and HDL-C/apoA-I, respectively.



**Figure 2**—HRs and 95% CIs for uptake of pharmacologic glucose therapy. Results are displayed as the effect of 1 SD lower baseline value of HDL-C (A) and HDL-C/apoA-I (B), respectively. Results are cumulatively adjusted for age and sex, then HOMA-IR, BMI, HbA<sub>1c</sub>, triglyceride (Trigs) levels, and, lastly, also for the potential confounders of LDL-C, waist circumference, hypertension, ALT, eGFR, the presence of micro- or macroalbuminuria, female menopause status, current smoking, regular alcohol consumption, and physical exercise.

did not materially change either the cross-sectional or prospective results.

## CONCLUSIONS

In our large study of predominantly Caucasian subjects with established type 2 diabetes, we report the novel observation that lower levels of HDL-C and a lower HDL-C/apoA-I ratio predict substantially earlier initiation of pharmacologic glucose control among those initially treated with lifestyle measures alone. This is in spite of no statistically significant baseline cross-sectional associations between HDL-related measures and HbA<sub>1c</sub> levels. The inverse association of progression to pharmacologic glucose control with HDL-C/apoA-I, which estimates HDL size, persists after adjustment for multiple metabolic and lifestyle factors. The results are thus consistent with the emerging concept that HDL biology may play a direct role in the development and progression of type 2 diabetes.

Our prospective findings need to be considered in light of our cross-sectional analysis which did not find hypothesized associations of HDL-C, apoA-I, or HDL-C/apoA-I with HbA<sub>1c</sub> or HOMA-B. There was no inverse correlation between HbA<sub>1c</sub> levels and HDL-related measures, despite low HDL-C being more common in type 2 diabetic patients than in the general population (3). Two smaller studies of type 2 diabetic patients in Italy and Saudi Arabia have previously reported modest but statistically significant inverse associations between HDL-C and HbA<sub>1c</sub> ( $r = -0.183$ ,

$P < 0.05$ ; and  $r = -0.074$ ,  $P < 0.005$ , respectively) (19,20). In both studies, participants appeared to have higher average HbA<sub>1c</sub> and HDL-C levels and longer diabetes duration than subjects in our study. Although our cohort is typical of an early type 2 diabetic population, it was selected for the purposes of a clinical trial. Lipid values formed part of the selection criteria and thus may have limited variability in HDL-C at baseline. Among all subjects who were screened for the trial ( $n = 13,900$ ; HDL-C range 0.29–3.47 vs. 0.45–2.96 mmol/L in the randomized cohort), there was a small inverse correlation between HDL-C and HbA<sub>1c</sub> ( $r = -0.030$ ;  $P < 0.001$ , age- and sex-adjusted). Furthermore, residual insulin secretion and intensity of glycemic therapy are the primary determinants of concurrent glycemia. Thus, important determinants of long-term glycemia may not be reflected in cross-sectional analyses (1). Although insulin resistance is a major determinant of the development and progression of type 2 diabetes (1), HOMA-IR predicted ~1% of the variation in HbA<sub>1c</sub> at baseline in our cohort ( $R^2 = 0.009$ ;  $P < 0.001$ ).

Our cross-sectional study also found no significant associations of HDL-C, apoA-I, or HDL-C/apoA-I with HOMA-B after adjustment for age, sex, and HOMA-IR. HOMA-B is positively correlated with HOMA-IR ( $r = 0.514$ ;  $P < 0.001$ ), since higher insulin resistance drives greater compensatory insulin secretion in early type 2 diabetes (1,27). Furthermore, HOMA-IR is inversely associated with HDL-related measures in the current study. This necessitates

adjusting associations of HOMA-B for variation in HOMA-IR (17). A very small inverse association between apoA-I and HOMA-B ( $R^2 = 0.001$ ;  $P = 0.001$ ) emerged after adjustment for HOMA-IR and all other available confounders. Although we cannot explain this association, it appears to be too small to be of clinical relevance. Notably, a cross-sectional study of 22 type 2 diabetic patients found no significant associations between HOMA-B and HDL-C or apoA-I, despite finding that HDL-related reverse cholesterol transport and antioxidant potential were positively associated with HOMA-B (17).

The progressive nature of type 2 diabetes warrants investigation of factors that may influence the rate of glycemic deterioration. The finding that lower HDL-C and HDL-C/apoA-I values portend earlier initiation of pharmacologic glucose control suggests that HDL metabolism may be contributory. For FIELD trial participants treated with lifestyle alone at baseline, the median time from diabetes diagnosis to need for pharmacotherapy was 6 years, a full year earlier among those in the lowest HDL-C/apoA-I quartile, and a full year later for those in the highest quartile, despite similar glycemic control and management throughout the study. The results accord with multiple studies showing that HDL-related measures predict incident type 2 diabetes (6–10,28). A single smaller study has previously reported a relationship between HDL-C and initiation of OHAs in established type 2 diabetes. It considered 705 patients who had an HbA<sub>1c</sub> ≤ 7% (HbA<sub>1c</sub> ≤ 53 mmol/mol) and

were using lifestyle measures alone at baseline (29). In unadjusted analyses, baseline HDL-C levels were lower among those who progressed to OHAs or whose HbA<sub>1c</sub> levels rose >7% at the end of 1 year.

Similarly, we have used the progression from lifestyle measures to pharmacologic glucose lowering as an index of worsening glycemia. During the trial, HbA<sub>1c</sub> <7.0% (53 mmol/mol) was the accepted target, and first OHAs were instituted at a median HbA<sub>1c</sub> of 7.1% (54 mmol/mol) in FIELD subjects (23,30). Nevertheless, the study protocol did not dictate glycemic management; hence, the possibility would exist that an association between low baseline HDL-C levels and initiation of first glycemic therapy might reflect more aggressive prescribing related to perceived low HDL-C-mediated risk. In order to exclude this possibility, we examined whether baseline HDL-C (or HDL-C/apoA-I) levels influenced either the HbA<sub>1c</sub> levels at which pharmacotherapy was commenced or total HbA<sub>1c</sub> rises over 5 years. Neither analysis supported such an alternative explanation. Also, lower baseline HDL-C and HDL-C/apoA-I predicted both earlier metformin and sulphonylurea uptake, suggesting that our results were independent of the mode of pharmacotherapy used (results not shown).

Our longitudinal analysis is adjusted for multiple lifestyle and metabolic factors, which could influence relationships between HDL-related measures and type 2 diabetic progression. Low physical activity, alcohol nonconsumption, and smoking are lifestyle factors that are associated with lower HDL-C and multiple other metabolic alterations (31,32). These factors did not materially affect the predictive value of HDL-C or HDL-C/apoA-I in our cohort. Similarly, the Prevention of Renal and Vascular End-stage Disease (PREVEND) study recently reported that both HDL-C and HDL-C/apoA-I predicted incident type 2 diabetes independently of current smoking and alcohol consumption (8). In the Diabetes Prevention Program (DPP), on-study HDL-C rise was associated with less progression to type 2 diabetes in the intensive lifestyle, metformin, and control arms (28).

In contrast to lifestyle factors, metabolic factors, in FIELD, partially account for the observed relationship between HDL-related measures and pharmacologic

glucose control initiation. After controlling for HOMA-IR and triglycerides, the HRs for both HDL-C and HDL-C/apoA-I were attenuated, and only HDL-C/apoA-I remained a significant predictor of progression to OHAs or insulin. Elevated insulin resistance and triglycerides are both independently and inversely associated with reduced HDL-C and HDL/apoA-I (33). Both factors are also thought to promote islet cell stress and apoptosis via associations with systemic low-grade inflammation and increased free fatty acid levels (27,34,35). Higher triglyceride levels and larger VLDL particles have also been reported to predict incident type 2 diabetes (9,10). However studies diverge as to whether HDL- or triglyceride-related measures are stronger predictors of incident disease. Importantly, the PREVEND study recently reported that both HDL-C and HDL-C/apoA-I predicted incident type 2 diabetes independent of triglyceride levels or HOMA-IR (8).

Our study thus provides further support for the notion that smaller cholesterol-poor HDL particles, reflected by lower HDL-C levels and HDL-C/apoA-I ratios, may contribute directly to worsening glycemia. Alteration to HDL size and cholesterol content may reflect reduced efficacy of reverse cholesterol transport in type 2 diabetes (36,37). Since HDL-mediated reverse cholesterol transport has been positively associated with insulin secretion and broader anti-inflammatory effects, this could provide a link between a reduced HDL/apoA-I ratio and more rapid deterioration in glycemic control (14,18,38).

Strengths of our study include a large sample size and the ability to account for multiple possible confounders, including lifestyle factors, metabolic factors, and statin and ACEi/ARB use. The length of follow-up and the high rate of therapy initiation enabled us to derive median times to event, which has not been possible in studies of diabetes incidence. The study has some limitations. Caucasian ethnicity was reported by 93% of subjects, which may limit the generalizability of the results. Lifestyle factors, which can influence HDL metabolism, were self-reported and may be affected by underreporting. Unless underreporting was differential with respect to HDL, this would only increase random error rather than introducing bias. Recording of

escalation of diabetes therapy could contain inaccuracies; however, any possible misclassification would result in dilution of potential associations with HDL-related parameters rather than the converse. This substudy was not a stated purpose of the FIELD trial; however, all analyses were performed after a hypothesis and prespecified analysis plan were prepared. One of the FIELD inclusion criteria was a total-cholesterol-to-HDL-C ratio >4. This may have some bearing on the range and spread of HDL-related measures in our cross-sectional analysis but, if anything, would have led us to underestimate our significant longitudinal associations. HOMA model estimates are not as reliable as those using clamp methods and could not be carried out on all subjects. However, they are a useful proxy in the context of a large cohort (39). HDL function and size were not directly measured in this study, and HDL-C and apoA-I may not reflect HDL function well (14,17,37). However, HDL-C is the most studied HDL-related measure and predictive of cardiovascular and microvascular complications in type 2 diabetes (4,5,40). ApoA-I is widely available, and elucidating its predictive value in type 2 diabetes is important. The HDL-C/apoA-I ratio is easy to derive, reasonably approximates HDL particle size, and has been previously linked to incident type 2 diabetes (8,21). Nonetheless, large clinical studies evaluating the associations of HDL particle number and function with concurrent and long-term glycemia in established type 2 diabetes would be of interest.

In summary, lower levels of baseline HDL-C and HDL-C/apoA-I were not cross-sectionally associated with HbA<sub>1c</sub>, but predicted earlier initiation of pharmacologic glucose control over 5 years in people with pre-existing type 2 diabetes. In particular, the value of HDL-C/apoA-I, an estimate of HDL particle size, in predicting glycemic progression was independent of all measured confounders. This provides clinical support for a direct involvement of HDL biology in worsening glycemia in people with established type 2 diabetes.

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**Author Contributions.** B.W. designed the current study, conducted the data analysis, interpreted the results, and drafted the manuscript. A.J.J. designed the current study, interpreted the data, and drafted the manuscript. T.M.E.D. and M.-R.T. designed and conducted the FIELD study, contributed to data interpretation, and edited the manuscript. R.S. conducted the FIELD study, contributed to data interpretation, and edited the manuscript. R.L.O. maintained the FIELD database and conducted statistical analysis on the current study. V.J.G. supervised data analyses and contributed to interpretation of the results. M.K.C.N. designed the current study, contributed to data interpretation, and edited the manuscript. A.C.K. designed and conducted the FIELD study, designed the current study, and contributed to the data analysis and interpretation and writing of the manuscript. A.C.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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