The Effect of Intensive Glucose Lowering on Lipoprotein Particle Profiles and Inflammatory Markers in the Veterans Affairs Diabetes Trial (VADT)

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OBJECTIVE—Intensive glucose-lowering therapy (INT) did not reduce macrovascular events in the recent randomized trials, possibly because it did not improve or worsen other traditional or novel cardiovascular risk factors.

RESEARCH DESIGN AND METHODS—Standard plasma lipids, cholesterol content of lipoprotein subfractions, and plasma inflammatory and prothrombotic markers were determined in a subgroup of the Veterans Affairs Diabetes Trial (VADT) participants (n = 266) at baseline and after 9 months of INT or standard therapy.

RESULTS—INT lowered glycated hemoglobin (by a median of 2% vs. a median of 0.7% by standard treatment; $P < 0.0001$); increased BMI (4 vs. 1%; $P < 0.001$), total HDL (9 vs. 4%; $P < 0.05$), HDL2 (14 vs. 0%; $P = 0.009$), LDL2 (36 vs. 1%; $P < 0.0001$), and plasma adiponectin (130 vs. 80%; $P < 0.01$); and reduced triglycerides (−13 vs. −4%; $P = 0.02$) and small, dense LDL4 (−39 vs. −13%; $P < 0.001$), but had no effect on levels of plasma apolipoproteins B-100 and B-48, C-reactive protein, interleukin-6, lipoprotein-associated phospholipase A2, myeloperoxidase, fibrinogen, and plasminogen activator inhibitor 1. Incident macrovascular events were associated with baseline interleukin-6 (hazard ratio per each quartile increase 1.33 [95% CI 1.06–1.66]), total LDL (1.25 [1.01–1.55]), apolipoprotein B-100 (1.29 [1.01–1.65]), and fibrinogen (1.26 [1.01–1.57]) but not changes in any cardiovascular risk factors at 9 months.

CONCLUSIONS—INT was associated with improved adiponectin, lipid levels, and a favorable shift in LDL and HDL subfractions after 9 months. These data suggest that the failure of INT to lower cardiovascular outcomes occurred despite generally favorable changes in standard and novel risk factors early in the study.

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Increased concentrations of small, dense lipoprotein particles, elevated levels of prothrombotic factors, and chronic low-grade inflammation are associated with insulin resistance and hyperglycemia and may contribute to increased risk of atherosclerosis and clinical macrovascular events (1–8). In several recent large randomized trials, intensive glucose-lowering therapy (INT) did not significantly decrease cardiovascular outcomes in type 2 diabetes, suggesting that targeting glycemic control alone is insufficient (9–11). One possible explanation is that intensive glucose lowering did not improve, or perhaps worsened, other relevant cardiovascular risk factors (10,12).

This study evaluated the effect of intensive glucose lowering on lipoprotein classes and subclasses, apolipoproteins B-100 and B-48, and concentrations of inflammatory and prothrombotic markers in the Risk Factors, Atherosclerosis and Clinical Events in Diabetes (RACED) substudy of the Veterans Affairs Diabetes Trial (VADT) (13). The VADT was a randomized trial comparing the effects of intensive and standard glucose lowering on new clinical macrovascular events in 1,791 military veterans who had a suboptimal response to therapy for type 2 diabetes (10). A 9-month follow-up interval was chosen for collection of blood for analysis of novel risk factors. This allowed us to capture changes in risk factors after glycemic control had stabilized (approximately 6 months into the VADT) and provided the opportunity to assess the prospective association between changes in traditional and novel cardiovascular risk factors and subsequent cardiovascular events (10).

RESEARCH DESIGN AND METHODS

A detailed description of the VADT and RACED substudy designs with inclusion and exclusion criteria has been published previously (10,13). Briefly, the primary treatment goal of the VADT was to achieve a 1.5% difference in glycated hemoglobin (HbA_{1c}) between those randomized to INT versus standard therapy while achieving optimal levels of other conventional cardiovascular risk factors. Both study groups were started on two oral agents: metformin plus rosiglitazone (if BMI ≥27 kg/m^2) or glimepiride plus rosiglitazone (if BMI <27 kg/m^2). Patients in the INT group were started on maximum medication doses, and those in the standard group were started on half maximum doses; the dose was titrated in both groups as needed to attain glycemic goals. Insulin was added for patients in the INT group who did not achieve an HbA_{1c} level of <6% and for those in the

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standard therapy group with a level of <9%. The primary VADT outcome was the time to the first occurrence of any one of a composite of macrovascular events, including myocardial infarction or stroke; death from cardiovascular causes; new or worsening congestive heart failure; surgical intervention for cardiac, cerebrovascular, or peripheral vascular disease; inoperable coronary artery disease; and amputation for ischemic gangrene. The RACED study included 324 patients with type 2 diabetes who were participating at seven VADT study sites. The RACED study was approved by the institutional review boards at these sites, and all participants gave written informed consent. The current analysis includes 266 RACED participants who had available measurement of plasma lipid subclasses and/or other novel risk markers at baseline and ~9 months after randomization.

Cholesterol within individual lipoprotein subfractions was quantified using the vertical autoprofile II technique (Atherotech, Inc., Birmingham, AL), as previously described (14,15). Briefly, lipoprotein classes [HDL, LDL, lipoprotein (a), IDL, and VLDL]; LDL subclasses (LDL1–4, with LDL1 being most buoyant and LDL4 most dense); and HDL subclasses (HDL2 and HDL3, with HDL2 being more buoyant) were separated by a single vertical spin density gradient ultracentrifugation at 416,000*g* for 36 min. After separation, lipoprotein classes and subclasses were collected continuously from the bottom of the centrifuge tube into the flow analyzer, where they react sequentially with a cholesterol-specific enzymatic reagent, producing a cholesterol concentration-dependent lipoprotein absorbance curve monitored by a spectrophotometer. The absorbance curve was further deconvoluted to provide cholesterol concentrations of lipoprotein classes and subclasses. The vertical autoprofile II method also defines the LDL density pattern based on the LDL maximum time, that is, the relative position of the LDL peak in the density gradient on a scale of 0–200 s, with 0 corresponding to the beginning of the HDL peak maximum and 200 corresponding to the VLDL peak maximum. Pattern A (predominantly large and buoyant LDL subclass) is defined by an LDL cholesterol peak occurring after 118 s, pattern B (predominantly small and dense LDL) is defined by an LDL cholesterol peak occurring before or at 115 s, and intermediate pattern (A/B) is defined by an LDL cholesterol peak occurring after 115 and before or at 118 s.

Commercial ELISA kits were used to measure serum concentrations of C-reactive protein (CRP; α Diagnostic International, San Antonio, TX) and interleukin-6 (IL-6; R&D systems, Minneapolis, MN). Serum adiponectin concentration was measured with a radioimmunoassay kit from Linco Research (St. Charles, MO). Lipoprotein-associated phospholipase A2 (LpPLA2) concentration was measured by a microplate-based ELISA (PLAC test, diaDexus, South San Francisco, CA). A high-sensitivity sandwich ELISA was used to measure serum myeloperoxidase (MPO) levels (PrognostiX, Cleveland, OH). Fibrinogen and plasminogen activator inhibitor 1 (PAI-1) concentrations were measured by established automated methods at the clinical laboratory at Tufts University (Boston, MA). Serum apolipoprotein (Apo) B-100 and ApoB-48 concentrations were measured by an established two-step sandwich chemiluminescent immunoassay using antibodies against the N-terminal of ApoB-100 and COOH-terminal of ApoB-48 (16) in a subset of 221 participants.

Statistical analyses were performed using software from the SAS Institute (version 9.2; Cary, NC). Wilcoxon signed rank test was used to determine differences from baseline within each group. A Kruskal-Wallis test was used for the comparisons between the two groups, and χ^2 and McNemmar tests were used for between- and within-group comparisons of category outcomes, respectively. Relationships between continuous variables were tested by partial Spearman correlation controlling for group assignment. General linear regression analysis, adjusted for group assignment, was used to identify potential effects of glucose-lowering drugs and other potential confounders on study outcomes. Cox proportional hazard risk analysis was used to determine the association between traditional and novel cardiovascular risk factors and development of macrovascular events occurring over the duration of the VADT. When examining the effect of 9-month change in risk factors, macrovascular events occurring before the blood was drawn at ~9 months were censored. *P* values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

There were no significant differences between the groups in baseline age (standard therapy vs. INT: median 61 vs. 60 years), race (71 vs. 65% white), sex (95 vs. 93% men), diabetes duration (median 10 years [for both]), and history of cardiovascular event (38 vs. 36%). Baseline metabolic characteristics and prothrombotic and inflammatory markers were similar within treatment groups, except for slightly higher levels of HDL and HDL3 cholesterol and lower IL-6 and PAI-1 with INT (Table 1).

Changes in medication use and concentrations of risk factors in blood after 9 months

After 9 months in the VADT, the INT group showed greater use of insulin, while both groups had higher use (but not statistically different rates between groups) of rosiglitazone and statins (Supplementary Table 1). Greater reductions in HbA_{1c} and fasting plasma glucose levels occurred with INT by design and were consistent with changes reported in the overall VADT population (Fig. 1*A*). Increases in BMI and HDL and reductions in triglyceride concentrations were greater with INT (Fig. 1*A*). There was a trend for greater reductions in ApoB-100 concentrations in the INT group ($P = 0.06$ vs. standard therapy; Fig. 1*A*).

The increases in larger LDL2 and HDL2 and the decreases in smaller LDL4 were greater with INT, whereas the standard group showed a significant increase in the largest LDL1 (Fig. 1*B*). The prevalence of LDL pattern B (characterized by a predominance of small, dense LDL) decreased in both groups, with a trend for greater declines with INT (from 88 to 64% INT, $P < 0.0001$; from 84 to 74% standard therapy, $P = 0.002$; $P = 0.06$, intensive vs. standard). The INT group also showed a smaller rise in VLDL, while IDL increased similarly in both groups and lipoprotein(a) trended higher in the INT group ($P = 0.16$ vs. standard) (Fig. 1*B*).

Plasma CRP and fibrinogen concentrations were reduced, and plasma concentrations of MPO, PAI-1, and adiponectin were increased in both groups (Fig. 1*C*). The standard group showed a significant increase in LpPLA2 levels (Fig. 1*C*). Adiponectin concentrations increased more with INT (Fig. 1*C*).

Table 1—*Metabolic characteristics and inflammatory markers at baseline by treatment group*

	Standard therapy	INT	*P* value
BMI (kg/m^2)	32 (28–35)	31 (29–35)	0.7
HbA_{1c} (%)	9.0 (8.2–10.2)	8.8 (8.1–10)	0.5
HbA_{1c} (mmol/mol)	75 (66–88)	73 (65–86)	
Fasting glucose (mg/dL)	200 (149–248)	185 (148–241)	0.4
Triglycerides (mg/dL)	182 (115–275)	151 (115–212)	0.3
ApoB-48 (mg/dL)	0.56 (0.29–0.91)	0.49 (0.31–0.86)	0.6
ApoB-100 (mg/dL)	126 (97–158)	131 (99–171)	0.4
Total cholesterol (mg/dL)	170 (151–191)	174 (154–198)	0.2
VLDL cholesterol (mg/dL)	24 (19–29)	22 (19–27)	0.3
IDL cholesterol (mg/dL)	11 (6–14)	9 (5–16)	0.3
Lipoprotein(a) cholesterol (mg/dL)	5 (4–9)	6 (4–9)	1.0
LDL cholesterol (mg/dL)	104 (93–124)	108 (93–131)	0.3
LDL1 (mg/dL)	15 (12–17)	15 (12–18)	0.3
LDL2 (mg/dL)	9 (5–15)	9 (6–14)	0.9
LDL3 (mg/dL)	39 (27–50)	43 (31–54)	0.06
LDL4 (mg/dL)	23 (12–32)	20 (14–29)	0.8
HDL cholesterol (mg/dL)	38 (32–44)	41 (35–46)	0.04
HDL2 (mg/dL)	7 (5–9)	7 (6–10)	0.4
HDL3 (mg/dL)	31 (27–36)	34 (29–38)	0.02
CRP (mg/L)	3.1 (1.6–6.3)	3.1 (1.3–6.7)	0.8
IL-6 (pg/mL)	3.3 (2.2–5.1)	2.8 (2.0–4.2)	0.02
LpPLA2 (ng/mL)	294 (238–350)	292 (241–351)	1.0
MPO (pmol/L)	657 (359–1,631)	824 (412–1,934)	0.2
Adiponectin (mg/L)	5.0 (2.9–8.2)	5.1 (3.4–8.4)	0.3
PAI-1 (units/mL)	40 (27–69)	32 (19–53)	0.04
Fibrinogen (mg/dL)	356 (305–398)	349 (296–398)	0.3

Data are presented as medians (25th–75th percentiles). Lp, lipoprotein.

Relationships of changes in novel cardiovascular risk factors with changes in traditional risk factors and with rosiglitazone dosage

Among the whole group, after controlling for group assignment, increases in LDL2 and reductions in LDL4 correlated with reductions in HbA_{1c} and triglycerides and with increases in total, LDL, and HDL cholesterol (Table 2). Increases in adiponectin concentrations correlated with reductions in triglycerides and increases in total and HDL cholesterol (Table 2). In multivariate analyses, changes in LDL2 and LDL4 levels were significantly associated with changes in HbA_{1c} (β coefficient −4.7 [standard error 1.6] and 0.5 [0.2], respectively) even after adjustment for changes in triglycerides, HDL, and group assignment.

Significant changes in LDL2, LDL4, and adiponectin with INT persisted after accounting for the use of glucose-lowering medications (data not shown), but they were significantly associated with rosiglitazone use in a dose-dependent manner (Fig. 2) even after adjustment for changes in HbA_{1c}, triglycerides, or HDL. There were significant correlations (controlled for group assignment) between changes in adiponectin and changes in both LDL2 ($r = 0.24$, $P = 0.0001$) and LDL4 ($r = -0.25$, $P < 0.0001$). Changes in both LDL2 and LDL4 were not associated with rosiglitazone dose after adjustment for changes in adiponectin ($P = 0.07$ and $P = 0.15$, respectively).

Association between risk factors and cardiovascular events in the VADT

Over the median 5 years of VADT follow-up, 76 participants from this cohort developed a new macrovascular event (after a median 2.4 years of follow-up [range 0.1–5.8 years]). The risk of developing the events was associated with higher baseline concentrations of total LDL, ApoB-100, IL-6, and fibrinogen (Table 3). No changes in traditional or novel cardiovascular risk factors during the first 9 months were associated with subsequent macrovascular events (Table 3).

CONCLUSIONS—In this study, assignment to INT led to a shift toward a less atherogenic lipid profile with increased HDL cholesterol; decreased triglyceride and ApoB-100 concentrations; larger, more buoyant LDL and HDL particles; and a greater increase in plasma adiponectin concentrations. On the other hand, compared with standard therapy, INT did not improve plasma concentrations of several inflammatory and prothrombotic markers previously shown to predict progression of atherosclerosis, occurrence of cardiovascular events, or both (2–4,6,7).

Increased prevalence of small, dense LDL particles seems to be related to poor glycemic control in type 2 diabetes (17). Previous studies also have shown an increase in LDL particle size after treatment with certain categories of diabetes medications (18–24). However, this is the first large, randomized study to examine the effect of intensive glucose lowering on lipoprotein particle profiles as well as other novel risk factors. We found a significant reduction of the smallest and perhaps most atherogenic LDL4 particles. Increased atherogenic potential has been ascribed to these particle because they seem to have reduced affinity to LDL receptors, increased propensity for transport into the vascular wall, increased binding to arterial wall proteoglycans, and augmented susceptibility to oxidative modifications (25). The reduction in LDL4 was accompanied by increases in larger, more buoyant LDL2 particles, so total plasma LDL cholesterol remained unchanged. The reciprocal change in LDL2 and LDL4 is in agreement with the concept of a shared but inversely related pathway for LDL2 and LDL4 production (26). The significant association of changes in LDL2 and LDL4 with change in triglyceride levels supports the concept that triglyceride metabolism is a key determinant of LDL subclass distribution (19,23). In fact, the strength of small, dense LDL as a predictor of cardiovascular outcomes in many clinical trials has been substantially diminished after taking into account traditional lipid categories, including triglycerides (6,7,23). In type 2 diabetes, cholesteryl esters seem to be preferentially transferred from HDL to small, dense LDL by the action of cholesteryl ester transfer protein (27). The correlation between declines in LDL4 cholesterol and increases in HDL cholesterol found in our study may indicate specific attenuation of cholesteryl ester transfer protein–mediated

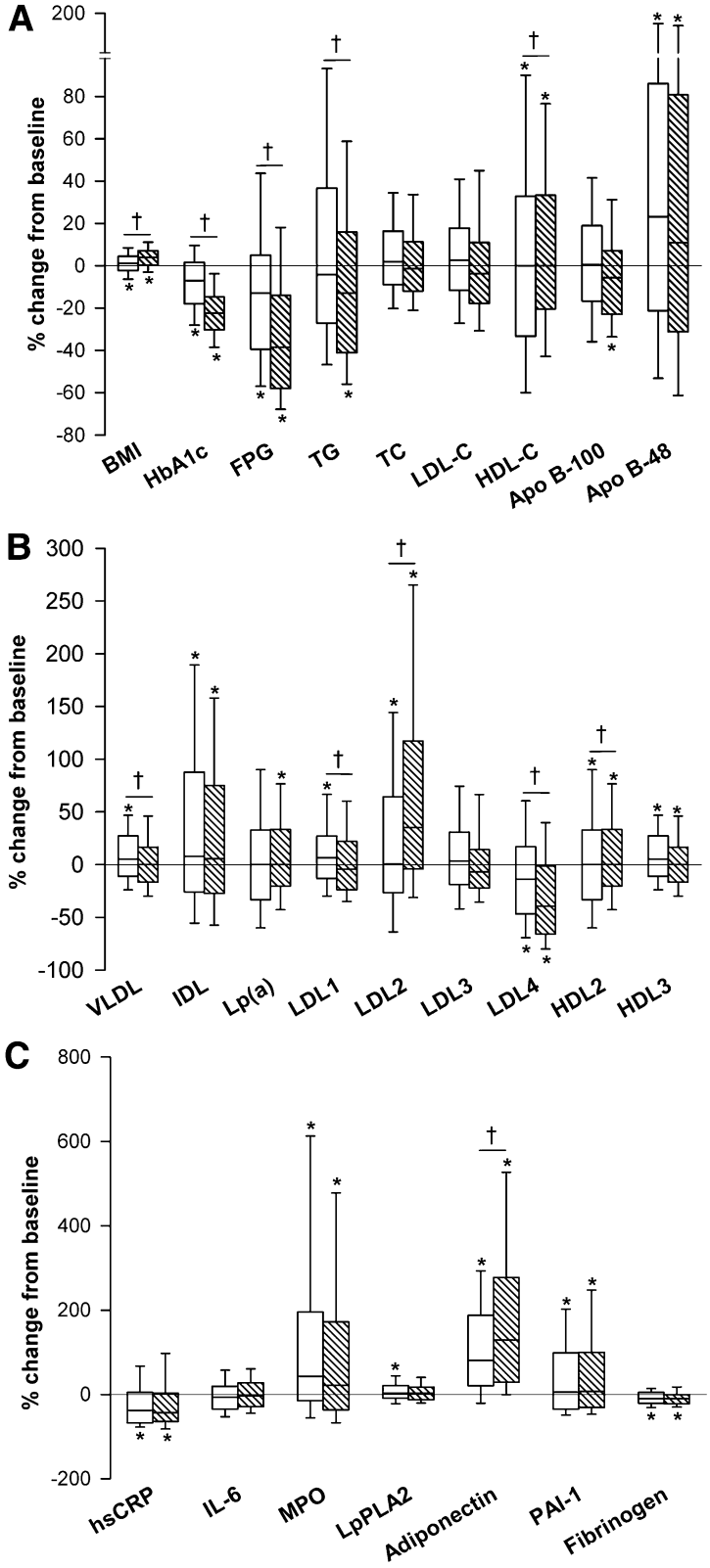

Figure 1—*Changes in BMI, glycated hemoglobin (HbA_{1c}), fasting plasma glucose (FPG), traditional plasma lipid categories (triglycerides [TG] and total cholesterol [TC]), apolipoproteins (Apo) B-100 and B-48* (A)*; lipid subclasses* (B)*; and inflammatory and prothrombotic markers* (C) *after 9 months of standard* (□) *or intensive* (▨) *glucose-lowering therapy. Box plots indicate (top to bottom) 95th, 75th, 50th, 25th, and 5th percentiles.* *$P < 0.05$, *9 months vs. baseline;* †$P < 0.05$, *intensive vs. standard. Lp(a), lipoprotein a; IDL, intermediate-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; MPO, myeloperoxidase; LpPLA2, lipoprotein-associated phospholipase A2; PAI-1, plasminogen activator inhibitor 1.*

cholesteryl ester transfer from HDL to small, dense LDL.

Although triglyceride lowering might have accounted for a substantial portion of the observed effect of intensive glycemic control, the independent association of LDL2 and LDL4 changes with changes in HbA_{1c} suggest a contributory role for lowering glucose in modulating the LDL density profile. It is important that changes in LDL2 and LDL4 were also independent of the use of specific classes of glucose-lowering medications, including insulin or rosiglitazone, that previously were shown to increase LDL particle size (18–21,24,28). In contrast with some previous studies (18,19,24), changes in LDL subclasses in our cohort were not related to insulin use. However, this association may have been missed because of the relatively low number of participants who started insulin therapy after randomization. On the other hand, the changes in LDL2 and LDL4 were greater with increasing doses of rosiglitazone. Interestingly, the association between rosiglitazone use and changes in LDL2 or LDL4 was not accounted for by changes in triglyceride or HDL cholesterol levels, but was attenuated after adjustment for changes in adiponectin concentrations. This is in agreement with a recent report showing that improvement of lipoprotein particle composition in patients with type 2 diabetes who are treated with pioglitazone was related to changes in adiponectin levels (29). A potential role for adiponectin in modulating distribution of lipoprotein subclasses is further supported by cross-sectional studies of population-based cohorts (30,31) and by studies of individuals with genetically based adiponectin deficiency (32). Of note, adiponectin has been shown to accelerate reverse cholesterol transport and decrease secretion of triglycerides from hepatic cells in vitro (33,34).

Both CRP and IL-6 predict atherosclerosis and cardiovascular events in general populations and in patients with diabetes (3,4). In this study, higher baseline IL-6 concentrations, but not CRP concentrations, in plasma were associated with incident macrovascular events independent of subsequent glucose therapy. Because CRP levels were quite elevated at baseline in both groups, this may have limited the ability to detect an association with incident cardiovascular disease. While both standard therapy and INT were associated with similar

Table 2—*Spearman correlation (adjusted for treatment assignment) of 9-month percent changes in LDL and HDL cholesterol subclasses and inflammatory and prothrombotic markers with 9-month changes in BMI, HbA_{1c}, and traditional plasma lipid categories*

	BMI	HbA_{1c}	TG	TC	LDL	HDL
LDL1	0.03	0.11	0.19*	0.83*	0.85*	0.14*
LDL2	−0.10	−0.18*	−0.28*	0.23*	0.21*	0.38*
LDL3	0.07	0.08	0.04	0.66*	0.72*	0.12
LDL4	0.07	0.25*	0.46*	0.27*	0.29*	−0.27*
HDL2	−0.13	0.01	−0.14	0.21*	0.10	0.74*
HDL3	−0.20*	−0.01	−0.32*	0.26*	0.15*	0.95*
CRP	0.13	0.07	−0.08	−0.05	0.00	−0.22*
IL-6	0.03	−0.02	−0.15*	−0.18*	−0.13	−0.20*
LpPLA2	0.10	−0.04	0.21*	0.49*	0.50*	0.01
MPO	0.11	0.03	0.03	0.00	−0.03	0.05
Adiponectin	0.07	−0.13	−0.16*	0.20*	0.12	0.39*
PAI-1	0.15*	−0.01	0.17*	0.05	0.04	−0.10
Fibrinogen	0.14	0.11	−0.11	−0.01	0.03	−0.07

TC, total cholesterol; TG, triglycerides. *$P < 0.05$.

decreases in plasma CRP concentrations, plasma IL-6 levels remained unchanged regardless of the treatment assignment. These findings are consistent with those reported in individuals with new-onset type 2 diabetes who received either placebo or glucose-lowering therapy with insulin, metformin, or both (12). The levels of the neutrophil activation marker MPO and the procoagulation factor PAI-1 were increased significantly after 9 months in both groups in our study, while the levels of LpPLA2, a marker of vascular inflammation, were significantly increased in the standard group only. The greater increase in adiponectin concentrations and lower LpPLA2 concentrations may indicate a modest anti-inflammatory effect of intensive glycemic control. In a smaller subgroup of this cohort, we have previously shown that lower LpPLA2 concentration at baseline predicted less progression of calcified atherosclerosis in coronary arteries over an average of 4.6 years (35). In contrast, the current analysis showed no association between LpPLA2 mass and clinical cardiovascular events. It has been suggested that with insulin resistance and diabetes, LpPLA2 mass and activity are more weakly related than in nondiabetic populations and may even show a divergent association with cardiovascular risk (36,37). Therefore, it remains to be established whether similar changes in LpPLA2 activity would occur with glucose lowering.

Despite marked differences in glycemic control, the risk of cardiovascular events with INT was not significantly reduced in the VADT (10), its RACED subset (13), or two other trials of patients with type 2 diabetes at relatively high risk for cardiovascular events (i.e., the ADVANCE [Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation] and ACCORD [Action to Control Cardiovascular Risk in Diabetes] studies) (9,11). Based on the current data in the RACED subset of the VADT, it seems likely that INT in these other studies may also have been associated with reduced levels of several inflammation-related risk factors and a shift toward less atherogenic plasma lipids and lipoprotein particle density profiles. This makes the failure of INT to reduce cardiovascular events in these studies even more surprising.

One may speculate that changes in these novel risk markers may have been too modest, or that these novel factors have a more limited effect on cardiovascular risk in patients with type 2 diabetes who have advanced atherosclerosis and are at a high risk for new or recurrent cardiovascular events (13). This notion seems to be supported by the presence of few associations between baseline levels or 9-month changes of these factors with subsequent cardiovascular events in our cohort. Moreover, in a previous study we showed that, except for LpPLA2 mass, there was no association between the baseline levels of many of these inflammation-related factors or traditional lipid categories and progression of calcified atherosclerosis (35). It is also possible that minimal or unfavorable changes in BMI, MPO, LpPLA2, PAI-1, or other novel risk factors may counteract the benefits of intensive glycemic control. For example, as suggested by the positive correlation between changes in BMI and PAI-1, weight gain might have offset favorable effects of lowering triglycerides on PAI-1 levels. Similarly, increased

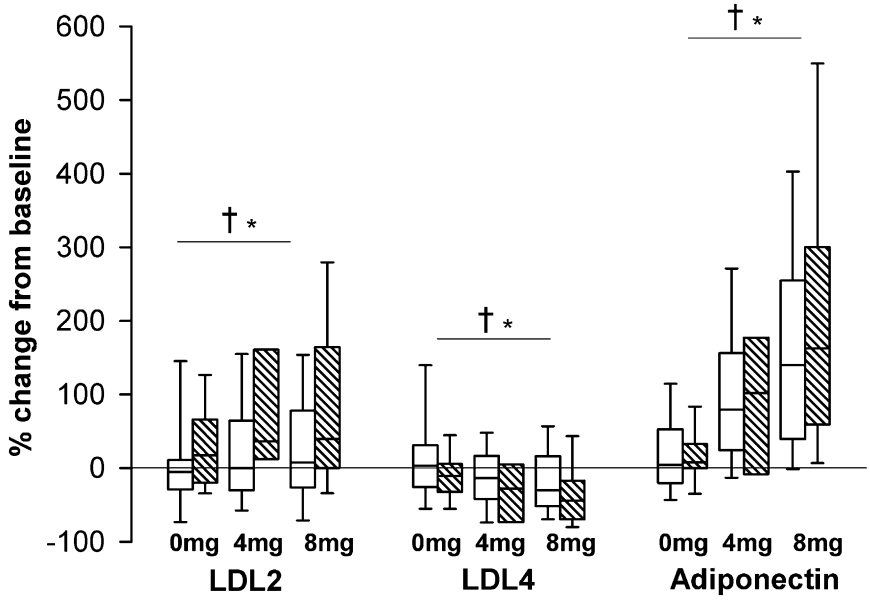

Figure 2—*Changes in LDL2, LDL4, and adiponectin by rosiglitazone dose and glucose-lowering therapy assignment (rosiglitazone 0 mg:* n = *27 [standard] and 25 [intensive]; 4 mg:* n = *55 and 5; 8 mg:* n = *55 and 99). Box plots indicate (top to bottom) 95th, 75th, 50th, 25th and 5th percentiles. *P < 0.05, rosiglitazone dose; †P < 0.05: intensive (▨) vs. standard (□).*

Table 3—*Hazard ratios (95% CIs) for new macrovascular events per each quartile increases in baseline levels and 9-month change of traditional and novel cardiovascular risk factors*

	Baseline	9-month change
Events (*n*)	76	62
BMI	1.03 (0.84–1.26)	1.19 (0.94–1.50)
Triglycerides	0.97 (0.79–1.18)	0.98 (0.78–1.22)
Total cholesterol	1.1 (0.89–1.36)	1.01 (0.81–1.27)
ApoB-48	1.06 (0.84–1.33)	0.99 (0.77–1.29)
ApoB-100	1.29 (1.01–1.65)*	0.98 (0.75–1.27)
LDL cholesterol	1.25 (1.01–1.55)*	1.0 (0.79–1.26)
LDL1	1.22 (0.99–1.51)	1.0 (0.8–1.25)
LDL2	0.94 (0.76–1.16)	0.93 (0.73–1.18)
LDL3	1.14 (0.92–1.41)	0.93 (0.73–1.18)
LDL4	1.03 (0.85–1.26)	0.96 (0.76–1.20)
HDL cholesterol	0.90 (0.73–1.12)	1.08 (0.86–1.35)
HDL2	0.89 (0.73–1.09)	1.26 (1.0–1.59)
HDL3	0.88 (0.71–1.10)	0.99 (0.79–1.24)
CRP	1.16 (0.94–1.43)	0.99 (0.79–1.24)
IL-6	1.33 (1.06–1.66)*	0.93 (0.73–1.17)
LpPLA2	0.98 (0.79–1.21)	1.09 (0.87–1.37)
MPO	1.06 (0.86–1.31)	0.92 (0.74–1.16)
Adiponectin	1.22 (0.99–1.50)	0.98 (0.77–1.23)
PAI-1	0.86 (0.69–1.08)	1.24 (0.96–1.61)
Fibrinogen	1.26 (1.01–1.57)*	1.13 (0.90–1.41)

*Values are adjusted for previous event and treatment assignment.

MPO (occurring in both treatment groups) may have offset elevations in HDL cholesterol levels by depriving HDL of its protective action against atherosclerotic plaques (38).

One caveat to this study is that measurement of changes in novel risk factors within the first year after randomization does not guarantee similar changes throughout the study. For example, by design, initial treatment-induced differences in triglyceride and HDL levels disappeared in the full VADT cohort after the median 5.6 years of follow-up (10). It is possible that the convergence of these standard lipid levels between treatment groups also lessened differences in novel risk factors over time. However, as glycemic control remained substantially different between treatment groups throughout the study and seemed to have an independent effect on LDL subclasses and other novel risk markers, some changes in novel risk factors caused by INT may have persisted. Moreover, the median time to cardiovascular event within this cohort was 2.4 years, suggesting that changes in risk factors measured within the first 9 months would be temporarily relevant to the development of subsequent cardiovascular events in our cohort. In addition, it seems improbable that the novel risk factors might have worsened in the INT group in the relatively short time interval between the 9-month time point and incident cardiovascular events. A second potential study limitation is that although we measured changes in many of the best-documented novel risk markers, there are undoubtedly other less well-recognized or yet unidentified novel factors that could be relevant to these results. If these unmeasured factors were not improved or were possibly worsened by INT, they could conceivably counteract the many observed benefits of intensive glycemic control.

In conclusion, the failure of INT to lower cardiovascular outcomes does not seem to be explained by the worsening of lipid subfractions and inflammation- or thrombosis-related cardiovascular risk factors. On the contrary, most of these risk factors showed more favorable early changes with intensive glucose control. These results make the failure of INT to decrease cardiovascular events in the VADT even more perplexing.

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J.K. researched data, contributed to discussion, and wrote the manuscript. A.S. researched data and contributed to data interpretation. G.B. contributed to data analysis and edited the manuscript. S.Y. contributed to analysis and interpretation of data. P.D.R. designed the study, obtained funding, contributed to discussion, and reviewed and edited the manuscript. J.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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