Antisense-Mediated Lowering of Plasma Apolipoprotein C-III by Volanesorsen Improves Dyslipidemia and Insulin Sensitivity in Type 2 Diabetes

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OBJECTIVE

To determine the effects of volanesorsen (ISIS 304801), a second-generation 2′-*O*-methoxyethyl chimeric antisense inhibitor of apolipoprotein (apo)C-III, on triglyceride (TG) levels and insulin resistance in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

A randomized, double-blind, placebo-controlled trial was performed in 15 adult patients with type 2 diabetes (HbA_{1c} >7.5% [58 mmol/mol]) and hypertriglyceridemia (TG >200 and <500 mg/dL). Patients were randomized 2:1 to receive volanesorsen 300 mg or placebo for a total of 15 subcutaneous weekly doses. Glucose handling and insulin sensitivity were measured before and after treatment using a two-step hyperinsulinemic-euglycemic clamp procedure.

RESULTS

Treatment with volanesorsen significantly reduced plasma apoC-III (−88%, *P* = 0.02) and TG (−69%, *P* = 0.02) levels and raised HDL cholesterol (HDL-C) (42%, *P* = 0.03) compared with placebo. These changes were accompanied by a 57% improvement in whole-body insulin sensitivity (*P* < 0.001). Importantly, we found a strong relationship between enhanced insulin sensitivity and both plasma apoC-III (*r* = −0.61, *P* = 0.03) and TG (*r* = −0.68, *P* = 0.01) suppression. Improved insulin sensitivity was sufficient to significantly lower glycated albumin (−1.7%, *P* = 0.034) and fructosamine (−38.7 μmol/L, *P* = 0.045) at the end of dosing and HbA_{1c} (−0.44% [−4.9 mmol/mol], *P* = 0.025) 3 months postdosing.

CONCLUSIONS

Volanesorsen reduced plasma apoC-III and TG while raising HDL-C levels. Importantly, glucose disposal, insulin sensitivity, and integrative markers of diabetes also improved in these patients after short-term treatment.

Insulin resistance states usually seen in the context of obesity and type 2 diabetes are commonly associated with a metabolic dyslipidemia that amplifies cardiovascular disease risk. Among patients with type 2 diabetes, insulin resistance impairs the ability to utilize glucose as fuel, prompting a switch toward fat storage that promotes free fatty acid flux, hepatic triglyceride (TG) synthesis, and secretion of large VLDL particles (1). Cholesteryl ester transfer protein-mediated exchange of

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VLDL-TG for cholesteryl esters in LDL and HDL particles promotes atherogenic small-dense LDL and dysfunctional small-dense HDL particles, which are more readily catabolized by the kidney. The resulting metabolic dyslipidemia features a triad of high levels of VLDL-TG, low HDL cholesterol (HDL-C), and an increased prevalence of small-dense LDL particles despite normal or even low LDL cholesterol (LDL-C) levels. The cumulative result is a significant increase in the risk of atherosclerosis (2).

Recommended treatments include diet and exercise as well as statins that have the strongest evidence for improvement of this condition (3–5). Because the TG-reducing effect of statins is modest at best, patient-centered guidelines also recommend a second drug such as fibric acid derivatives, niacin, or n-3 fatty acids (3). In support of this approach, meta-analyses of five large trials assessing the impact of fibrates on cardiovascular end points revealed benefits among patients with the lipid triad (6).

While it is well established that insulin resistance drives dyslipidemia, there is growing evidence that the elevation in plasma TGs and reduction in HDL-C accompanying insulin resistance states may exacerbate the insulin-resistant phenotype, albeit by yet-to-be-defined mechanisms (7). In preclinical models, HDL may improve insulin sensitivity, insulin secretion through the β-cells, and β-cell survival (8). Clinically, elevations in TG-rich lipoprotein (TRL) levels have been shown to exacerbate the pathology of insulin resistance. Therefore, reducing TG and increasing HDL may not only reduce cardiovascular risk but also improve insulin sensitivity and delay β-cell failure (1,9).

Apolipoprotein (apo)C-III has been identified as a key modulator of plasma TG concentrations in animal models and in humans. ApoC-III affects TG metabolism by inhibiting lipoprotein lipase (LPL) activity and by interfering with receptor-mediated uptake of TRLs by the liver (10,11). Mice overexpressing apoC-III not only have high plasma TG but also display increased insulin resistance (12). In East Indian Asians, an *APOC3* genotype associated with increased plasma TG is also associated with insulin resistance and nonalcoholic fatty liver disease, the hepatic manifestation of the metabolic syndrome (13). However, in more recent studies, it does not appear that apoC-III is directly associated with the incidence of nonalcoholic fatty liver disease in broader patient populations (14). Two recent Mendelian randomization studies showed that individuals with loss-of-function *APOC3* variants had lower TG concentrations and LDL-C, higher HDL-C, and, compellingly, a 40% reduction in risk of coronary heart disease (15,16). While it is unclear whether the coronary heart disease risk reduction is due to suppressed TG, other TRL-associated factors, or apoC-III per se, the aforementioned cardioprotective properties suggest that apoC-III is an attractive therapeutic target (17).

Potent second-generation antisense oligonucleotides (ASOs) have been developed to reduce apoC-III expression in animals and humans (18). In mice, administration of a species-specific apoC-III ASO led to dose-dependent reductions in hepatic apoC-III mRNA and plasma apoC-III protein. In dyslipidemic rodent models, this led to >50% reductions in both fasting and postprandial plasma TG. Recapitulating preclinical models, treating normolipidemic and hypertriglyceridemic human subjects with the human-specific apoC-III ASO, volanesorsen (ISIS 304801), profoundly reduced plasma apoC-III protein and plasma TG levels (18,19). This included subjects with familial chylomicronemia syndrome (FCS), who have extreme hypertriglyceridemia due to loss-of-function mutations leading to absent LPL activity (20). This latter study demonstrates that apoC-III also inhibits an LPL-independent pathway of TRL clearance.

Given evidence that hypertriglyceridemia may worsen insulin sensitivity and the data suggesting a role for apoC-III in mediating insulin resistance, we reasoned that an intervention that robustly suppressed apoC-III and plasma TG would lead to improved insulin sensitivity. Thus, we conducted a clinical trial that allowed us to test the mechanistic hypothesis that aggressively lowering TG by apoC-III ASO treatment would improve insulin sensitivity in patients with diabetes with high TGs.

RESEARCH DESIGN AND METHODS

Clinical Trial Design and Participants

A randomized, double-blind, placebo-controlled, two-step hyperinsulinemic-euglycemic (HE) clamp trial was conducted at a single center in the U.S. (Profil Institute for Clinical Research, San Diego, CA) between 8 August 2012 and 31 January 2014 to evaluate the effects of volanesorsen treatment in adult patients with type 2 diabetes and hypertriglyceridemia. The primary objective of this trial was to determine the effects of volanesorsen versus placebo on fasting total apoC-III. The secondary objectives were to determine its effects on other lipid parameters, glycemic control, and insulin sensitivity. Written informed consent was obtained from all participants prior to participation in the study. The protocol was approved by the institutional review board and conducted in compliance with the standards of Good Clinical Practice and guidelines of the 2002 Declaration of Helsinki.

Eligible patients were 18–65 years old; had hypertriglyceridemia (TG >200 mg/dL and <500 mg/dL) and type 2 diabetes that had been diagnosed for at least 6 months; had uncontrolled hyperglycemia (HbA_{1c} >7.5% [58 mmol/mol]); and were on a stable dose of metformin ≥1,000 mg/day. Patients with uncontrolled high blood pressure, heart failure New York Heart Association III and IV, recent episode of angina, prior acute myocardial infarction, recent coronary bypass surgery, severe diabetes microvascular complications, kidney or liver disease, hypothyroidism, or low platelet count were excluded from participation in the study. Acceptable contraceptive methods were required during dosing and in the follow-up period. Lipid and blood pressure medications were allowed provided that they were stable before dosing. Oral antidiabetes medication other than metformin was prohibited. Men had to restrict alcohol intake to 2 drinks and women to 1 drink daily. All patients received glucose-monitoring instructions and dietary education based on the American Diabetes Association guidelines.

The trial consisted of three periods (Supplementary Fig. 1): screening, dosing (13 weeks, days 1–85), and post-treatment/follow-up (13 weeks, days 86–176). Eligible patients were randomized 2:1 to receive volanesorsen 300 mg: placebo for a total of 15 subcutaneous doses (days 1, 3, 5, 8, 15, 22, 29, 35, 42, 49, 56, 63, 70, 78, and 85). On the

mornings of days 1 and 92, patients underwent a two-step HE clamp procedure.

Volanesorsen is a second-generation 2′-*O*-methoxyethyl chimeric ASO designed to sequence-specifically reduce expression levels of the human apoC-III mRNA (18).

Pharmacodynamics

The primary pharmacodynamic (PD) variable was fasting plasma apoC-III concentrations. Secondary PD variables included whole-body insulin sensitivity measurements including those that affect insulin sensitivity (nonesterified fatty acids [NEFA]), integrative markers of glucose control (glycosylated albumin, fructosamine, glycosylated HbA_{1c}), fasting total TG, and other lipoproteins, including VLDL–apoC-III, total cholesterol, LDL-C, HDL-C, VLDL cholesterol (VLDL-C), apoB, and non-HDL-C. ApoC-III and lipids were measured by MedPace Reference Laboratories (Cincinnati, OH). ApoC-III was determined by rate nephelometry. HDL-C was determined after separation from apoB-containing lipoproteins (dextran sulfate precipitation); VLDL-C and LDL-C were determined after isolation by ultracentrifugation. Cholesterol content was determined using a standard enzyme-based colorimetric assay. Markers of glycemic control including those collected during the clamp procedure were measured by KineMed, Inc. (Emeryville, CA).

Safety Assessments

Safety assessments included treatment-emergent adverse events, vital signs and weight, clinical laboratory tests (LabCorp, San Diego, CA), physical examination, electrocardiogram, and use of concomitant medications.

Two-Step HE Clamp Procedure

A two-step HE clamp procedure was performed at baseline (day 1) and end of treatment (day 92) using the Biostator (MTB Medizintechnik, Amstetten, Germany). Insulin (Humulin R U100, Eli Lilly & Co., Indianapolis, IN) was infused using a precision pump at a rate calculated to acutely elevate serum insulin concentration from basal (fasting) levels to predefined plateaus (21,22).

On the evening prior to the clamp procedure, after the placement of intravenous forearm catheters, an overnight variable intravenous regular human insulin infusion was initiated to achieve a stable target glucose of 110 ± 10 mg/dL for at least 2 h prior to starting the clamp. At the initiation of the clamp, a low-dose insulin infusion of 30 mU/m^2/min (step 1) was started and maintained for 3 h. Dextrose 20% was variably infused to maintain the plasma glucose target of 110 mg/dL. After 3 h, the insulin infusion rate was increased to 150 mU/m^2/min (step 2) for an additional 3 h. Sampling for glucose and insulin occurred every 10 min for the last 30 min of each step during the steady-state plateau. The dose of insulin at the first step was chosen to avoid completely suppressing endogenous (principally hepatic) glucose production; the dose at the second step was chosen to provide a robust stimulus to glucose disposal, primarily in skeletal muscle.

The primary measure of whole-body insulin sensitivity was the insulin sensitivity index (SI_{clamp}). This index is based on the glucose infusion rate (GIR) per minute from the last 30 min of each step and the corresponding plasma concentrations of insulin and blood glucose:

$$SI_{clamp} = \frac{\text{mean(GIR)}_{\text{step 2}} - \text{mean(GIR)}_{\text{step 1}}}{\left[\text{mean(I)}_{\text{step 2}} - \text{mean(I)}_{\text{step 1}}\right] \times \text{mean(BG)}_{\text{steps 1 and 2}}}$$

where I is insulin and BG is blood glucose. Similarly, we used steady-state measures to calculate corroboratory parameters: *1*) the glucose disposal rate (M), the GIR corrected for body weight; *2*) the glucose metabolic clearance rate (MCR), 100 × (mean[M]/mean[BG]), and *3*) the glucose metabolism–to–insulin ratio (M/I): M divided by the insulin concentration, I.

Further details on these methods are available in the Supplementary Data.

Statistical Analysis

Sample size was based upon prior clinical experience with volanesorsen (18) and powered by 80% to detect a 45% difference in apoC-III levels between volanesorsen and placebo-treated groups at an α-level of 0.05, under the assumption of a 50% reduction in the volanesorsen treatment group and 5% in the placebo group.

The safety population consisted of all patients who were randomized and received at least one dose of study drug. The per-protocol population included patients who received at least nine doses of study drug, had a valid baseline total apoC-III plasma measure and at least one postbaseline measure, and did not have any significant protocol deviations that would be expected to bias the patients' PD assessments. The primary analysis was a comparison of the percent changes from baseline to day 91 in fasting total apoC-III levels in the volanesorsen group and placebo group of the per-protocol population. The data were analyzed using the Wilcoxon rank sum test.

Exploratory analyses were performed on the data collected from the HE clamp procedure ($SI_{clamp,}$ M, MCR, M/I). These mechanistic outcomes were analyzed by mixed effects regression, with treatment group, sequence (day 1, day 92), and their interaction as fixed effects and patient as a random intercept. Two patients were excluded from the analysis: one had a technical problem that invalidated the day 92 clamp procedure, and one did not complete the day 92 procedure. Four patients received fewer doses than planned, so a fixed covariate was included in the clamp regressions to account for suboptimal dosing. For sensitivity analyses, we used the Wilcoxon rank sum test. To measure the strength of the linear relationship between variables, we used the Pearson correlation coefficient.

RESULTS

Patients

Fifteen patients were randomized (5 placebo and 10 volanesorsen) in this study (Supplementary Fig. 2). The mean (SD) age was 56.5 (7.5) years, and 73% were women (Table 1). All patients were white, and all were overweight or obese (mean [SD] BMI 33.1 [4.4] kg/m^2). The mean (SD) baseline fasting TG level was 249.3 (70.2) mg/dL, and HbA_{1c} was 7.90 (0.62)% (63 mmol/mol).

Eleven of the 15 patients received all 15 doses of treatment (7 active and 4 placebo), 2 received 12 doses of active drug, 1 received 7 doses of active, and 1 received 7 doses of placebo. Dosing was discontinued by the sponsor for administrative reasons.

Volanesorsen Improves Metabolic Dyslipidemia in Patients With Type 2 Diabetes

Administration of volanesorsen resulted in a rapid and prolonged suppression of fasting plasma apoC-III concentrations

(Fig. 1*A*). A similar response was observed in TG levels (Fig. 1*B*). All treated patients achieved TG levels <105 mg/dL by 4 weeks of dosing, and at the end of treatment the average TG level was 75.9 mg/dL (Table 1). In addition, HDL-C increased (Fig. 1*C*) and non-HDL-C, a measure of all apoB-containing lipoproteins, decreased (Fig. 1*D*). After 13 weeks, compared with placebo, volanesorsen produced a statistically significant reduction from baseline in apoC-III (−87.5%, $P = 0.019$), TG (−69.1%, $P = 0.019$), and VLDL–apoC-III (−90.2%, $P = 0.023$) while raising HDL-C (42.5%, $P = 0.034$) (Table 1). Importantly, this marked decrease in TG was not associated with increases in LDL-C, while non-HDL-C and apoB decreased nonsignificantly. There were no changes in plasma NEFA levels ($P = 0.412$).

Volanesorsen Improves Insulin Sensitivity in Proportion to TG Suppression

Volanesorsen improved whole-body insulin sensitivity compared with placebo (Fig. 2 and Supplementary Table 1). Among those randomized to volanesorsen, SI_{clamp} improved 50%, compared with a 7% drop on placebo (adjusted difference 57%, $P < 0.001$ mixed model, $P < 0.05$ Wilcoxon rank sum test). Within the treated group, six out of eight patients (75%) displayed at least a 20% improvement in insulin sensitivity. We tested whether changes in SI_{clamp} depended on TG suppression (Fig. 3 and Supplementary Table 2) and found that TG suppression strongly correlated with improvements in SI_{clamp} ($r = -0.68$, $P = 0.01$). Improved whole-body insulin sensitivity also depended on suppression of other components that reflect TLR levels, including apoC-III ($r = -0.61$, $P = 0.03$), VLDL–apoC-III ($r = -0.61$, $P = 0.03$), and VLDL-C ($r = -0.66$, $P = 0.01$). The relationship between improved insulin sensitivity and total plasma apoC-III suppression is especially noteworthy as the primary effect of volanesorsen.

Moreover, at the high insulin rate, volanesorsen raised insulin-corrected glucose disposal (M/I) 30% compared with placebo ($P = 0.02$) (Supplementary Table 1). Though glucose disposal (M) and MCR each rose about 25% on volanesorsen, comparison with placebo was not statistically significant ($P = 0.08$ and $P = 0.06$, respectively).

Table 1—Baseline characteristics and effect of 300 mg volanesorsen on lipid and lipoprotein levels and glycemic control

	Placebo	Volanesorsen	*P*
Baseline characteristics			
N	5	10	
Sex, female:male	3:2	8:2	
Age, years	55.0 (10.0)	57.2 (6.4)	
BMI, kg/m^2	32.5 (4.9)	33.4 (4.4)	
Fasting glucose, mg/dL	180.2 (31.3)	180.9 (29.3)	
HbA_{1c}, %	7.6 (0.3)	8.0 (0.7)	
HbA_{1c}, mmol/mol	60.0 (3.7)	64.3 (7.6)	
TGs	215.2 (48.6)	266.3 (75.2)	
Fasting lipids and lipoproteins			
N	4	9	
ApoC-III			
Baseline, mg/dL	11.7 (2.3)	13.9 (4.4)	
Day 91, mg/dL	11.1 (3.4)	1.7 (0.6)	
% change	−7.3 (14.0)	−87.5 (5.4)	0.019
TGs			
Baseline, mg/dL	223.0 (52.3)	260.1 (77.0)	
Day 91, mg/dL	202.8 (71.1)	75.9 (18.6)	
% change	−9.9 (19.9)	−69.1 (10.1)	0.019
VLDL-C			
Baseline, mg/dL	41.9 (7.3)	49.5 (20.1)	
Day 91, mg/dL	36.8 (18.0)	12.1 (4.8)	
% change	−13.5 (40.8)	−72.9 (13.0)	0.059
VLDL–apoC-III			
Baseline, mg/dL	6.2 (2.4)	7.3 (3.1)	
Day 91, mg/dL	5.8 (2.5)	0.6 (0.4)*	
% change	−8.3 (20.4)	−90.2 (7.3)*	0.023
HDL-C			
Baseline, mg/dL	38.9 (6.6)	41.1 (7.7)	
Day 91, mg/dL	36.5 (11.4)	57.8 (13.3)	
% change	−7.2 (16.5)	42.5 (32.2)	0.034
LDL-C			
Baseline, mg/dL	146.5 (22.3)	122.1 (39.4)	
Day 91, mg/dL	138.5 (23.2)	118.2 (31.3)	
% change	−5.5 (7.2)	0.0 (26.3)	0.706
Non-HDL-C			
Baseline, mg/dL	188.4 (28.8)	171.6 (50.2)	
Day 91, mg/dL	175.3 (40.9)	130.3 (34.5)	
% change	−7.6 (13.8)	−22.1 (18.5)	0.168
ApoB			
Baseline, mg/dL	128.3 (17.3)	111.9 (31.6)	
Day 91, mg/dL	115.8 (25.1)	86.6 (18.5)	
% change	−10.4 (8.5)	−20.8 (15.9)	0.270
NEFA			
Baseline, mg/dL	0.5 (0.1)	0.7 (0.3)	
Day 91, mg/dL	0.6 (0.2)	0.6 (0.2)	
% change	20.0 (28.3)	−5.0 (36.8)	0.412
Glycemic control			
N	4	9	
Glycated albumin, %			
Baseline	15.6 (0.5)	16.2 (1.7)	
Day 91, change from baseline	0.7 (1.6)	−1.7 (1.2)	0.034
Day 176, change from baseline	1.8 (1.8)	−2.1 (2.4)	0.059
Fructosamine, μmol/L			
Baseline	244.3 (4.2)	273.8 (31.0)	
Day 91, change from baseline	14.5 (33.2)	−38.7 (22.5)	0.045
Day 176, change from baseline	47.8 (22.5)	−11.6 (22.6)	0.019
HbA_{1c}, %			
Baseline	7.8 (0.2)	7.9 (0.6)	
Day 91, change from baseline	0.50 (0.62)	−0.27 (0.50)	0.100
Day 176, change from baseline	0.78 (0.71)	−0.44 (0.39)	0.025

Continued on p. 1412

Table 1—Continued

	Placebo	Volanesorsen	*P*
HbA_{1c}, mmol/mol			
Baseline	61.5 (1.9)	62.7 (6.2)	
Day 91, change from baseline	5.5 (6.8)	−2.9 (5.4)	0.100
Day 176, change from baseline	8.5 (7.8)	−4.9 (4.2)	0.025

Data are means (SD) unless otherwise indicated. Baseline data represent the safety population (all randomized patients who received at least one dose of study drug); PD data represent the per-protocol population (patients who received at least 9 doses of study drug, had a valid baseline total apoC-III measure and at least one postbaseline measure, and did not have any significant protocol deviations that would be expected to bias the patients' PD assessments). Baseline is defined as the average of all assessments prior to the first dose. Primary end point is defined as the day 91 results. For patients who terminated treatment early, the primary end point is defined as the first measurement after last dose. *P* value determined by the Wilcoxon rank sum test. *n = 8 for these values.

Volanesorsen Improves Clinical Markers of Glycemic Control

Volanesorsen substantially improved integrative markers of glucose control, lowering glycated albumin (−1.73%, P = 0.034) and fructosamine (−38.7 μmol/L, P = 0.045) 1 week after the last dose (day 91). Moreover, volanesorsen lowered HbA_{1c} (−0.44%, P = 0.025) at the end of the follow-up period (3 months after the last dose) (Table 1). These changes correlated significantly with reductions in TG (r = 0.60, P = 0.03) and apoC-III (r = 0.60, P = 0.03) levels (Supplementary Table 2). Furthermore, there was a moderate percentage weight loss at the end of treatment with volanesorsen (−2.1%, P = 0.05 vs. placebo), which persisted to the end of the study (−3.6%, P = 0.04 vs. placebo) (Supplementary Table 3).

Tolerability and Adverse Effects

There were no deaths in this study or discontinuations of study drug due to an adverse event. There was one serious adverse event of syncope in the volanesorsen treatment group, which was moderate in severity and considered unlikely to be related to the study drug. The most common adverse events occurred at the injection site, followed by upper–respiratory tract infection (5 patients [50%]) and headache (5 patients [50%]). Overall, 15% of all injections were accompanied by local cutaneous reactions at the injection site. The majority of adverse events in both treatment groups were mild in severity. There were no clinically relevant changes in serum chemistries, hematology, urinalysis, inflammatory markers, electrocardiogram, or vital signs.

CONCLUSIONS

The administration of volanesorsen to patients with hypertriglyceridemia and poorly controlled type 2 diabetes on metformin led to a profound decrease in plasma apoC-III and TG levels, which was associated with a marked improvement in whole-body insulin sensitivity. The improvement in insulin sensitivity was strongly related to plasma apoC-III and TG suppression and was reflected in improved moderate (glycated albumin and fructosamine) and long-term (HbA_{1c}) indices of plasma glucose control. These data suggest that volanesorsen treatment improved both the dyslipidemia characteristic of type 2 diabetes and glucose control.

The effects of apoC-III inhibition on the metabolic dyslipidemia of patients with type 2 diabetes are supported by several studies. Patients with type 1 diabetes and type 2 diabetes display elevated apoC-III levels (23–26). Additionally, studies have shown good correlation between apoC-III levels and TG in various populations (27–29). The potential mechanism by which apoC-III inhibition may improve the metabolic dyslipidemia of these patients is thought

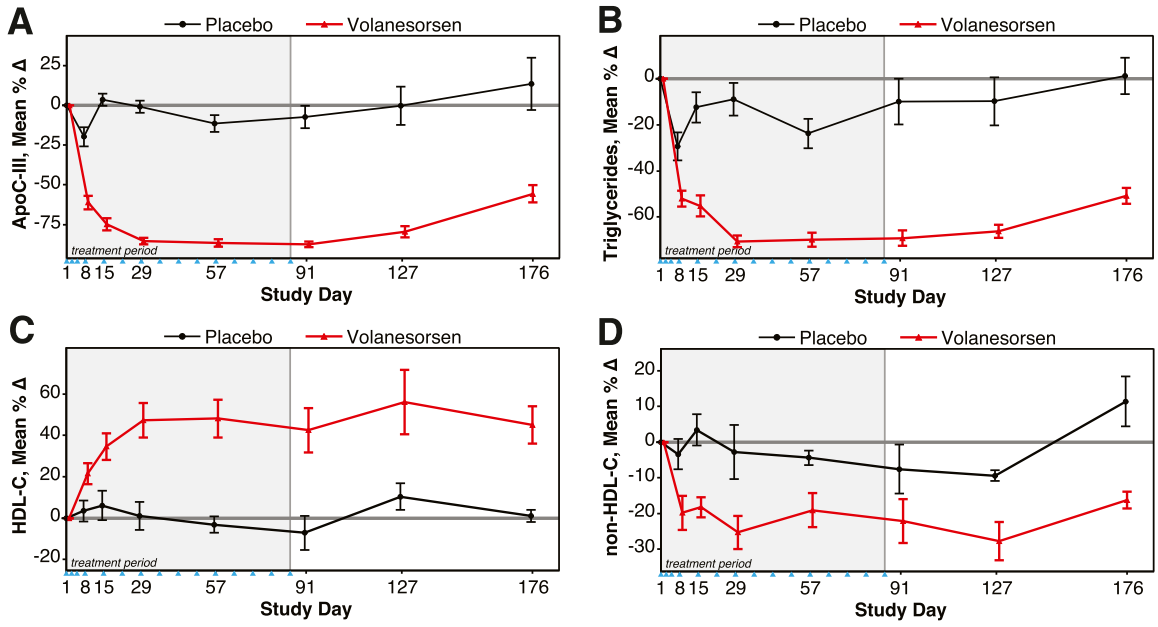

Figure 1—Effect of volanesorsen 300 mg on plasma apoC-III and lipid levels over time. ApoC-III (*A*), TGs (*B*), HDL-C (*C*), and non-HDL-C (*D*). Data are shown as the mean percentage change from baseline. Error bars represent the ± SEM. Solid blue triangles indicate dosing days.

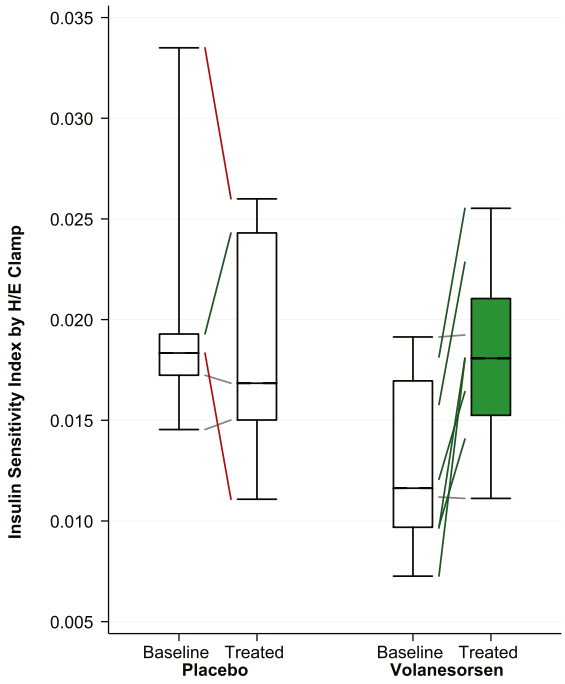

Figure 2—Volanesorsen 300 mg improves whole-body insulin sensitivity on clamp. In this box plot the horizontal line represents the median, the box represents the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentiles. The colored lines between baseline and treated clamps demonstrate the response of individual patients. A green line indicates a 20% or greater improvement in insulin sensitivity, whereas a red line indicates a 20% or greater worsening from baseline.

to be enhancement of LPL activity and enhanced hepatic uptake of TRL (10,11,20). Of note, the reduction in TG noted in this study was not associated with increases in LDL-C, as often seen with TG-lowering therapies. Furthermore, the reduction in TGs was associated with increases in HDL-C, presumably by reducing the cholesteryl ester transfer protein-mediated exchange of HDL-C for TRL TG (10).

The most intriguing finding of the current study was that profound suppression of apoC-III and TG levels by volanesorsen improved insulin sensitivity. Volanesorsen increased mean SI_{clamp} >50% over baseline and raised SI_{clamp} at least 20% in the large majority of patients. Such robust improvements are not typical for current approved TG-lowering medications. The concept that apoC-III, independently or via hypertriglyceridemia, worsens insulin sensitivity is supported in several ways by the results of the current study. First, a potent TG-lowering intervention enhanced insulin sensitivity compared with placebo. Second, there was a strong association between the decrement in TG and the increment in SI_{clamp} (r = 0.68, P = 0.01). Third, improved insulin sensitivity depended also on apoC-III suppression as a whole by the group-wise comparison. The presence of a strong relationship between improved whole-body insulin sensitivity and apoC-III suppression across individual patients strengthens the case that apoC-III suppression may be causally related to improved insulin sensitivity.

Moreover, the favorable effect on whole-body insulin sensitivity translated into improvements in medium and long-term glucose handling as reflected by significant plasma reductions in percent glycated albumin, fructosamine, and HbA_{1c} concentrations. Recent evidence indicates that, like HbA_{1c}, the medium-term markers also associate with cardiovascular risk (30,31). The reduction in HbA_{1c}, which reached statistical significance versus placebo 3 months after cessation of treatment, likely reflects the long drug elimination half-life of ~4 weeks (Supplementary Table 4) and subsequent prolonged duration of action.

Several lines of evidence have implicated elevated TG and their by-products in insulin resistance (1–3). Clinically, elevations in TRL levels have been shown to exacerbate the pathology of insulin resistance (9). It is thought that the inability of insulin resistant adipose tissue to store TG may be the initial step in the development of insulin resistance (1,31). There is molecular support for the concept that TGs provoke insulin resistance by peripheral catabolism in situ by tissues expressing LPL, causing local increases in NEFA uptake, resulting in excess intracellular fatty acid metabolites. Intracellular fatty acid accumulation is thought to disrupt insulin receptor substrate phosphorylation, ultimately impairing insulin receptor actions downstream, such as glucose transport (32). Reductions in peripheral venous NEFA levels were not observed in the patients in this study. The use of circulating NEFA as a biomarker is limited by evidence suggesting that plasma NEFA sampled downstream from the site of NEFA uptake does not necessarily predict intracellular NEFA accumulation. Clinical experiments have shown that excessive plasma NEFAs are not required for hypertriglyceridemia to provoke insulin resistance (33), and suppressing NEFA does not reverse insulin resistance when intracellular fatty acids remain elevated (34).

The expression of apoC-III is regulated by insulin via the insulin response element encoded in the *APOC3* gene (35–37). Plasma concentrations of apoC-III were previously found to be associated with the degree of insulin resistance as estimated by HOMA (38). ApoC-III inhibition may also enhance the sensitivity to the normal insulin-mediated suppression of *APOC3* gene expression (10), which is dysregulated in patients with type 2 diabetes. Finally, data also suggest that circulating apoC-III itself may act as a diabetogenic factor regulating the preservation of pancreatic β-cells by as yet undefined mechanisms (9).

A recent study showed that elevated apoC-III is associated with the hypertriglyceridemia seen in both generalized and partial lipodystrophy, suggesting that apoC-III may represent a therapeutic target in these patients (38). Patients with lipodystrophy, unable to store excess calories in adipocytes, develop ectopic lipid deposits in muscle and liver, which lead to an increase flux of free fatty acids and severe insulin resistance (39). Current therapies used to treat diabetes and severe insulin resistance (thiazolidinediones, U-500 insulin)

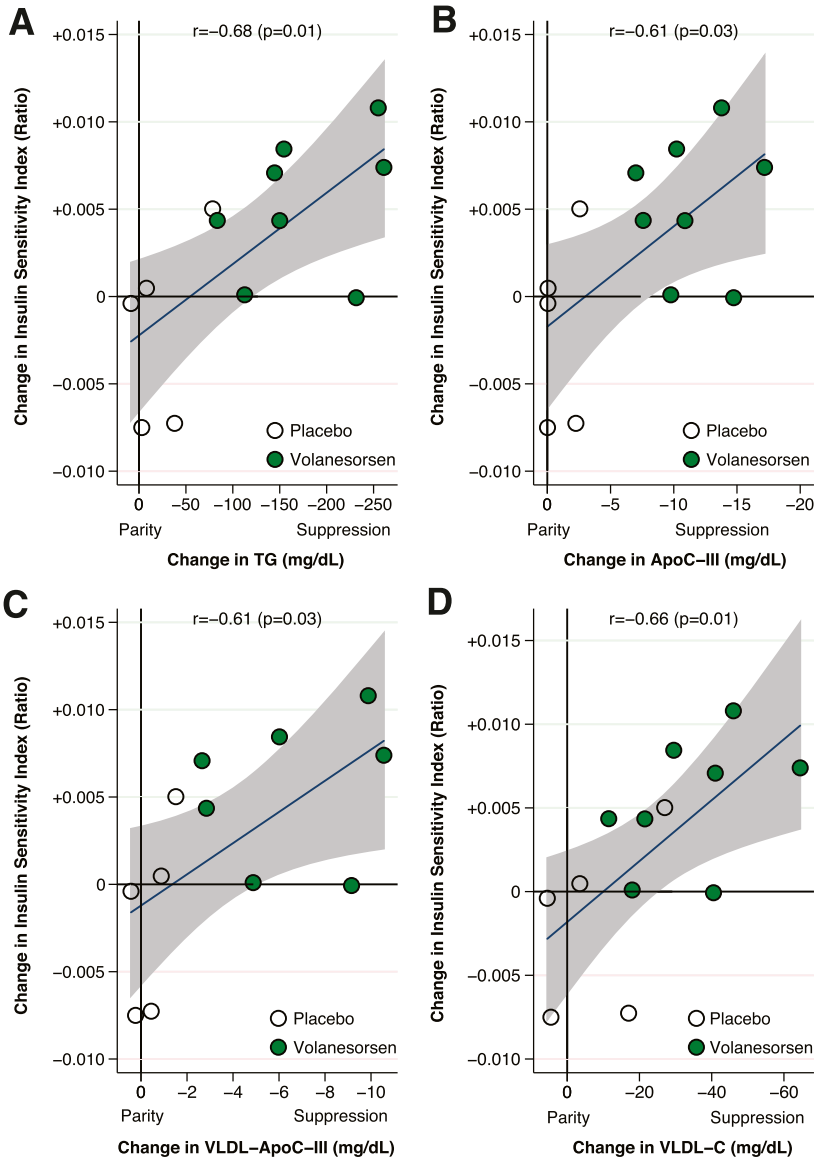

Figure 3—Improved insulin sensitivity depends on suppression of TRLs and apoC-III. Absolute change from baseline at end of treatment in TGs (*A*), apoC-III (*B*), VLDL–apoC-III (*C*), and VLDL-C (*D*) vs. posttreatment change in insulin sensitivity index. Shaded area represents the 95% CI.

and/or high TG (fibrates, niacin, fish oils) are not very efficacious in this patient population (40). By reducing TG levels and improving insulin sensitivity, apoC-III inhibition may improve the metabolic profile of patients with lipodystrophy, as well as those with FCS, reducing their risk of acute pancreatitis and other complications associated with diabetes and potentially of cardiovascular events. A recently started trial evaluating the effects of volanesorsen in patients with partial lipodystrophy, referred to as BROADEN (NCT02527343), and the placebo-controlled trial in patients with FCS, referred to as APPROACH (NCT02211209), will help answer some of the questions posed above.

Whether pharmacological inhibition of apoC-III could result in an improvement in cardiovascular outcomes is unknown but merits further consideration if we take into account that individuals with a loss-of-function mutation in *APOC3* have 40% lower TG levels and a 40% reduction in coronary artery disease risk (15,16). This consideration is further supported by the TG-dependent association found recently between apoC-III and coronary artery calcification, a measure of atherosclerosis, in patients with type 2 diabetes (27).

This study has several limitations. First, the data were obtained in a very small number of subjects and need to be replicated in a larger cohort. Therefore, at this stage these data should only be considered exploratory and hypothesis generating, which is especially true of the mechanistic clamp study. Second, the current clamp design does not provide insight into the mechanism of improved insulin sensitivity: hepatic glucose production versus peripheral glucose uptake. Further understanding of this mechanism would be of interest. On the one hand there is a mechanistic basis supporting the concept that profoundly suppressing circulating TGs might improve peripheral glucose uptake. On the other hand, an association between apoC-III and hepatic glucose production has been reported (12).

In conclusion, inhibition of apoC-III with a second-generation ASO improved the metabolic dyslipidemia of patients with type 2 diabetes. ApoC-III inhibition also improved whole-body insulin sensitivity and clinical markers of glucose handling. Improved insulin sensitivity was tightly correlated with TG suppression, consistent with the concept that hypertriglyceridemia exacerbates insulin resistance. These findings support further investigation to clarify the mechanism and better define the population that may benefit. Clinically, robust TG suppression by inhibition of apoC-III could complement diabetes management.

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Duality of Interest. A.D. is an employee of Akcea Therapeutics, a subsidiary of Ionis Pharmaceuticals, Inc. R.L.D. is an investigator for an ongoing volanesorsen clinical trial (clinical trial reg. no. NCT02211209). V.J.A., R.G.L., M.J.G., S.G.H., R.Y., W.S., B.F.B., S.B., and R.M.C. are employees of the study sponsor, Ionis Pharmaceuticals, Inc. M.H. and L.M. are employees of the study center, Profil Institute for Clinical Research, Inc. No other potential conflicts of interest relevant to this article were reported.

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W.S., and S.B. contributed to the study design. R.G.L., M.J.G., and R.M.C. provided the original concept. M.H. and L.M. acquired data. A.D., R.L.D., V.J.A., M.H., L.M., R.Y., W.S., B.F.B., and S.B. analyzed and/or interpreted data. All authors were involved in drafting and/or critical revision of the manuscript. A.D. 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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