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Lipid Profiles, Inflammatory Markers, and Insulin Therapy in Youth with Type 2 Diabetes

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Abstract

Objectives: Data regarding atherogenic dyslipidemia and the inflammation profile in youth with type 2 diabetes is limited and the effect of insulin therapy on these variables has not previously been studied in youth. We determined the impact of insulin therapy on lipid and inflammatory markers in youth with poorly controlled type 2 diabetes.

Study design: In the TODAY multi-center trial, 285 participants failed to sustain glycemic control on randomized treatment (primary outcome: HbA1c < 8% for 6 months); 363 maintained glycemic control (never reached primary outcome). Statins were used for LDL-cholesterol > 130

^{**}List of additional members of the TODAY study group is available at www.ipeds.com (Appendix).

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Materials developed and used for the TODAY standard diabetes education program and the intensive lifestyle intervention program are available to the public at <https://today.bsc.gwu.edu/>.

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mg/dL. Upon reaching primary outcome, insulin was started. Changes in lipids and inflammatory markers (slopes over time) were examined.

Results: Progression of dyslipidemia was related to glycemic control. In those with primary outcome, insulin therapy impacted HbA1c modestly, and dampened the rise in total cholesterol, LDL-cholesterol, and total apolipoprotein B, although statin use increased from 8.6% to 22% one year after primary outcome. The rise in triglycerides and plasma non-esterified fatty acids stabilized after insulin was started, independent of HbA1c. There was a rise in high-sensitivity C-reactive protein that continued after insulin initiation, related to HbA1c and percent overweight.

Conclusions: Worsening dyslipidemia and inflammation over time raise concern regarding premature development of atherosclerosis in youth with type 2 diabetes. Insulin therapy has a limited benefit in the absence of glycemic control. Strategies to achieve better glycemic control are needed.

Keywords

Adolescent; type 2 diabetes mellitus; lipids; insulin; inflammatory markers

The increasing prevalence of type 2 diabetes in youth is expected to contribute to a rise in diabetes-related complications, including cardiovascular disease (1,2). This concern is heightened by the high prevalence of cardiovascular risk factors and markers of cardiovascular end organ injury in this population (3). TODAY (Treatment Options for type 2 Diabetes in Adolescents and Youth) was a multicenter, randomized clinical trial ([ClinicalTrials.gov: NCT00081328](https://clinicaltrials.gov/ct2/show/study/NCT00081328)) designed to compare the effect of 3 treatment regimens to maintain glycemic control in youth with recent-onset type 2 diabetes. The TODAY cohort provides a unique opportunity to examine the effects of insulin therapy in youth with type 2 diabetes with poor glycemic control on metformin (rosiglitazone was stopped upon insulin initiation).

In light of the known accordance between glycemic control with dyslipidemia and markers of inflammation, we set out to explore the impact of reaching primary outcome with subsequent initiation of insulin upon these variables.

The objective of the current study was to determine the impact of insulin therapy on lipid profiles, and inflammatory markers in the TODAY participants who reached primary outcome. We hypothesized that insulin therapy would ameliorate lipid abnormalities and chronic inflammation in obese youth with type 2 diabetes, related to improved glycemic control.

METHODS

Details regarding the TODAY study design and methods have been reported (6,7). In brief, 699 youth aged 10–17 were enrolled between July 2004 and February 2009. Participants had type 2 diabetes of <2 years duration using criteria of the American Diabetes Association, a body mass index [BMI] ≥85th percentile for age and sex, and negative pancreatic autoantibodies. Patients with refractory hyperlipidemia (n=2): total cholesterol >300 mg/dL or LDL >190 mg/dL or triglycerides >800 mg/dL, despite appropriate medical therapy, were

excluded from participation in the study. After randomization, participants were seen every two months in the first year and quarterly thereafter. HbA1c was assessed at each visit and other laboratory measures including fasting lipid and inflammatory markers were determined at baseline, 6 months, and annually. The TODAY protocol was approved by the Institutional Review Boards at each participating institution. Parents of children and adolescents provided written informed consent; children and adolescents provided assent.

When participants reached primary outcome metformin was continued, rosiglitazone was discontinued in the M+R group and insulin was added. After initiation of insulin therapy, participants and clinicians remained masked to the original treatment assignment, but were unmasked to HbA1c. Initial insulin treatment was 0.2 units/kg glargine insulin each evening and was increased up to 1.0 units/kg/d (maximum 100 units) until fasting blood glucoses reached 70–150 mg/dL.

Lipid lowering medications [LLM], primarily atorvastatin, were initiated for persistent LDL-C levels ≥ 130 mg/dL or triglyceride levels 300–599 mg/dL after six months of nutrition and diabetes management per algorithm (6). If triglycerides were ≥ 600 mg/dL, fibrate therapy could be initiated at the discretion of the physician, in addition to invigorating measures to achieve glycemic control, given the known relationships between hypertriglyceridemia and hyperglycemia.

Of the 699 TODAY participants, 34 of the 319 who reached primary outcome were excluded from the preset analysis (12 had ≥ 1 full-term/pre-term pregnancies, 1 required multiple episodes of temporary insulin treatment, and 21 reached primary outcome but never received insulin, ie, reached primary outcome right before the end of the study or were lost to follow-up after reaching primary outcome). Comparison of these 21 excluded participants to the 285 included in the sample showed that those lost-to-follow up were more likely to be male (13 of the 21 or 62% were male vs. 37% among the 285, $P = .0250$) but were not found different with respect to age at baseline, race-ethnicity, baseline Tanner stage, SES (highest level of household education and income), duration of diabetes, baseline BMI or baseline HbA1c. 363 TODAY participants maintained glycemic control during the study and never started insulin.

All samples were shipped on dry ice to the Northwest Lipid Research Laboratory, University of Washington, Seattle, WA. The methods and analytical performance for determination of HbA1c, lipids, separation of LDL fractions, apoB in plasma and in LDL fractions, plasma non-esterified fatty acids [NEFA], hs-CRP, plasminogen activator inhibitor-1 [PAI-1], and homocysteine were previously described (6). Analysis of Interleukin-6 [IL-6] was performed using human high sensitivity magnetic beads-based method (EMD Millipore Inc., Billerica MA). The assay sensitivity was 0.18 pg/mL. The intra- and inter-assay CVs were 7% and 8%, respectively, for the low quality control samples and 6.8% and 8.4%, respectively, for the high quality control.

Height (cm) and weight (kg) were measured as previously described (6) and used to calculate BMI in kg/m^2 . Percent overweight, a weight-related metric measure now widely used for describing and tracking heavier children (8), was defined as BMI minus BMI at the

50th percentile for age and sex based on Centers for Disease Control and Prevention growth charts, divided by BMI at the 50th percentile, times 100.

Statistical analyses

Data are presented as mean and standard deviation or percent. Chi-square tests or t-tests were used to compare demographic and laboratory characteristics at 'time 0' between the TODAY participants who reached primary outcome and those who never reached it. For the group who reached primary outcome, 'time 0' was defined as the date long-term insulin therapy was started; for those who didn't reach primary outcome, 'time 0' was defined as the mid-point in the study, resulting in equal duration in the study in the two groups.

Piecewise random coefficient modeling was used (9,10), which allows for comparison of trends (slopes reflecting a change in outcome over time) corresponding to time before and time after a defined 'time 0'. This method is appropriate for repeated measures data collected at uneven time intervals and allows for covariate adjustment.

SAS PROC MIXED (statistical software version 9.3, SAS Institute Inc, Cary, North Carolina) was used to fit the piecewise random coefficient model. The model consisted of regressing each lipid or inflammatory marker outcome variable as a function of time relative to 'time 0,' thereby obtaining one intercept (at 'time 0') and two slopes (one before and one after 'time 0') for each participant. The two times (relative to 'time 0') were included in the model as random effects. Intraclass correlations coefficients (ICCs) were calculated for each random effects model. The ICCs obtained ranged from 55% to 71% for the lipid models and ranged from 45% to 74% for the inflammatory marker models, suggesting some degree of between-subject variance in the observations. The impact of HbA1c and percent overweight was also examined by evaluating models +/- HbA1c and/or percent overweight as time-varying covariates. Randomized treatment group was also considered as a covariate in our analysis but not retained in the final models because it was not found to be a significant contributor in any of the models. Data were included up to 3 years before and 3 years after 'time 0'. A parallel analysis was performed on the dichotomous outcome LDL particle density (cut-off at relative flotation rate 0.263) using SAS PROC GENMOD and testing for trends in percentages over time rather than slopes.

Extreme values in the distribution of each outcome (defined as <1st and >99th percentiles) were set to missing. Values were used even if the participants were placed on LLM or antihypertensive medications during the study. PAI-1, hs-CRP, homocysteine, IL-6, and triglycerides were log-transformed prior to modeling due to lack of normality. HDL-C analyses were performed separately for each sex. Interaction terms of subgroup variables of interest with the pre-insulin and post-insulin slopes were added to the models to assess differences by race-ethnicity, sex, and parental history of dyslipidemia or cardiovascular disease. All analyses were considered exploratory, and p-values <0.05 were considered statistically significant; the study was powered for the primary outcome only.

RESULTS

Participant demographic and laboratory characteristics at the time long-term insulin therapy was started ('time 0') in the group that experienced primary outcome are shown in Table I. These characteristics were compared with those from participants who maintained glycemic control and never started insulin, using data collected at an equivalent time point in the study as 'time 0'. Participants starting insulin therapy had higher levels of total cholesterol, triglycerides, apoB, apoB in LDL fractions, cholesterol in LDL fractions, PAI-1, hs-CRP, and IL-6 compared with those not requiring insulin.

As the changes in the lipid profiles and inflammatory markers over time in participants who never reached primary outcome (maintained glycemic control) have been previously reported (5), the remainder of the analyses focused on the group of participants who reached primary outcome and started insulin therapy during the study.

Total cholesterol, LDL-C, cholesterol in LDL fractions, and total apoB increased prior to insulin therapy (Figure 1, A-D). The rise continued after insulin initiation but at a reduced rate; slopes before and after starting insulin were significantly different (Table II). After adjustment for HbA1c and percent overweight, slopes were reduced and differences in slopes before vs. after insulin initiation were no longer significant. Triglycerides (Figure 1, E) rose prior to insulin therapy and stabilized after starting insulin; significance did not change with HbA1c and percent overweight in the model. HDL-C concentrations in females were increasing before 'time 0' and became flat after beginning insulin; slopes for HDL-C in males were uniformly flat (Figure 1, F and G). The slope for apoB in LDL fractions stabilized after 'time 0' (Figure 1, H). None of these changes were significant in the adjusted models. The ratio of apoB to cholesterol in LDL fractions was significantly different before vs. after 'time 0' in the unadjusted model only (Figure 1, I). Addition of HbA1c attenuated these relationships.

The percent of subjects with small dense LDL particles rose significantly from 60.5% at 1 year prior to 'time 0' to 75.9% at insulin start ($p<.0001$), and then stabilized after 'time 0' ($p=ns$). This increase prior to start of insulin was significant in the unadjusted model ($p=0.0077$) but not in the model adjusted for HbA1c.

In the unadjusted model, NEFA levels rose before insulin initiation and then flattened (change in slope before vs after significant, $p=0.0045$) (Figure 1 J and Table II). After adjusting for HbA1c and percent overweight, the difference in slopes before vs after 'time 0' was no longer significant.

PAI-1 and hs-CRP (Figure 2, A and B) concentrations were significantly rising before the start of insulin therapy. PAI-1 decreased after starting insulin therapy (significant negative slope) and slopes before vs. after 'time 0' were significantly different in both unadjusted and adjusted models (Table II). Hs-CRP continued to increase after insulin start, but the before vs after 'time 0' slopes were not significantly different in the adjusted model, after taking into account HbA1c and percent overweight. Homocysteine and IL-6 (Figure 2, C and D) rose significantly both before and after insulin initiation, but the differences were only significant for homocysteine in the adjusted model. Percent overweight was a significant

covariate ($p<.0001$) along with HbA1c in all inflammatory markers models except for homocysteine.

We further evaluated the effect of reduction in HbA1c on lipids and inflammatory markers after start of insulin therapy. After 6 months of insulin therapy, the decrease in HbA1c in those with primary outcome was approximately -0.11% (SD 2.1). 40.2% decreased their HbA1c by 0.5% and 27.6% of participants decreased their HbA1c by 1% . Analyses were then performed for 2 groups based on change in glycemia: those who achieved a 1% reduction in HbA1c after 6 months on insulin vs. those who did not (Table III; available at www.jpeds.com). Overall, the slopes for the lipid and inflammatory markers following insulin initiation decreased after insulin initiation in those with HbA1c reduction 1% . Those with less change in HbA1c showed a smaller decrease in cardiovascular disease risk markers following insulin initiation.

Use of prescribed LLM increased in those with primary outcome from 8.6% at 'time 0' to 22.0% 1 year later. The percentage of participants prescribed LLM showed an increasing trend before and up to 'time 0' versus a flat trend post 'time 0' (trend comparison pre vs post = $p<.0001$ unadjusted, $p=0.0029$ adjusted for HbA1c). A sensitivity analysis was performed excluding any participant who was prescribed a LLM at any time during the study. The main outcomes remained essentially unchanged (data not shown), raising the possibility of nonadherence with statin therapy in many participants.

Pre- ($p=0.0191$) and post-insulin ($p=0.0061$) slope differences by sex were found for hs-CRP, but the sex difference was similar across both time periods ('before' and 'after' insulin start), with males having overall lower trends over time compared with females, irrespective of insulin use. Lower levels of IL-6 over time were found in males compared with females but only prior to insulin start ($p=0.0069$). No differences by sex were found between the slopes for IL-6 after the start of permanent insulin. Similarly, a race-ethnicity difference in the slopes over time was found for LDL-C ($p=0.0065$) between Hispanic and non-Hispanic White, but only before the start of insulin (Table IV and Figure 3; available at www.jpeds.com). After start of permanent insulin, the LDL-C differences by race-ethnicity were no longer significant. No other sex or race-ethnicity differences between the slopes 'before' and 'after' insulin start were found for all other lipids, NEFA, PAI-1, and homocysteine ($p>0.05$).

No differences by parental history of dyslipidemia or cardiovascular disease were found for any of the lipids, NEFA, or the inflammatory markers, except for homocysteine. Pre-insulin ($p=0.0116$) slope differences by parental history of cardiovascular disease were found for homocysteine, but only prior to insulin start with lower levels of homocysteine found in participants without parental history of cardiovascular disease. No parental history differences were found between the slopes for homocysteine after the start of permanent insulin (data not shown).

DISCUSSION

Both HbA1c and dyslipidemia contribute to adverse changes in markers of subclinical atherosclerosis in youth with type 2 diabetes, including carotid intima media thickness and arterial stiffness (3,11–14). In youth without diabetes, progressive dyslipidemia typical of those with type 2 diabetes is associated with increased indicators of subclinical atherosclerosis in adulthood (15). Data regarding the atherogenic profile in youth with type 2 diabetes and the effect of insulin therapy in these youth are limited. In adults, type 2 diabetes alone is a risk factor for higher carotid intima media thickness (IMT) and worsening glucose control is associated with progression of subclinical atherosclerosis (16). The presence of cardiovascular risk factors, including higher LDL particle number, is associated with higher carotid IMT (17). Cardio-metabolic risk is also impacted by systemic inflammation, as indicated by levels of biomarkers of systemic inflammation, including hs-CRP, PAI-1 and Homocysteine. Of these, hs-CRP is a strong CVD risk predictor (18). Our current study demonstrates that levels of hs-CRP were related to change in HbA1c and rose before and after start of insulin therapy.

The results in this study show a close relationship between progression of dyslipidemia and glucose control. Although LDL particle number and size were not analyzed, the lipid changes described, including rise in triglycerides and apoB before initiation of insulin and increase in the percentage with smaller dense LDL particles, are consistent with higher atherogenic risk in association with poor diabetes control. The rise in NEFA prior to insulin therapy and its stabilization following insulin initiation is consistent with effects of insulin on NEFA release and clearance. Elevated NEFA leads to overproduction of VLDL particles and their subsequent remodeling after release from the liver (19). The initiation of insulin therapy appeared beneficial in dampening the rise in total cholesterol, LDL-C, apoB, and NEFA mainly through an effect on HbA1c, but the percentage with smaller LDL particles did not change, indicating a persistence of the shift to smaller, denser LDL particles. Overall, the observed changes in our study are consistent with short term clinical studies in adults supporting a favorable effect of insulin therapy on total and atherogenic LDL-C subgroups, when HbA1c is better controlled. With improvement in glycemia, a decrease in apoB secretion and stable or increasing HDL-C may be seen (20–23). The mechanisms of insulin action include an effect on up regulation of cholesteryl ester transfer protein in one study (24) and a decrease in large VLDL and hepatic lipase activity in another (22).

The rise in triglyceride concentrations prior to reaching primary outcome stabilized after insulin therapy, independent of HbA1c. This may reflect the effect of insulin on suppressing lipolysis. In support of this, a parallel change was observed in NEFA levels. This effect of insulin therapy on decreasing triglycerides is consistent with the findings in the intensive vs. standard insulin treatment arms of the Veterans Affairs Cooperative Study in type II Diabetes Mellitus (VA CSDM (24) and others (20). The beneficial effect of insulin on the lipid profile was limited, likely related to continued suboptimal glucose control despite insulin therapy, or to inadequate insulin exposure due to noncompliance. Consistent with this, there was a more marked overall improvement in the slopes for the lipids and inflammatory markers in the subset of participants that showed greater improvement in HbA1c at 6 months post insulin initiation. Simultaneously, the need for LLM increased over

time, consistent with worsening of the lipid profile. A main effect of glycemia on the lipids and inflammatory markers trends was verified by a sensitivity analysis that excluded participants who received LLM. This is again consistent with findings in the VA CSDM study where the lipid trends in the sub-cohort not receiving LLM were identical to those in the total study population (24). In addition, results from our sensitivity analysis raise concerns about non-compliance with LLM in these participants.

The combination of hyperglycemia and hyperlipidemia promotes atherogenesis (25), raising the question of whether insulin therapy needs to be intensified or started earlier in the course of youth-onset type 2 diabetes along with strategies to improve adherence. Intensive insulin therapy in adult clinical trials of new and poorly controlled diabetes generally show improvements in lipid profiles both in type 1 (26) and type 2 diabetes (20,24), including when combined with metformin (27). Long-term follow-up data in adults from the United Kingdom Prospective Diabetes Study show that insulin use was associated with 15% reduction in myocardial infarction, 24% reduction in microvascular disease, and 13% reduction in death in individuals with type 2 diabetes (28).

However, multiple trials (29–31) in adults with type 2 diabetes designed to examine the effect of early insulin therapy on cardiovascular events in older individuals (mean age 63.5 years) were not successful in establishing clear benefit (32). In contrast to the TODAY study, these protocols were designed to evaluate intensive insulin therapy in adults with type 2 diabetes and many participants already had a significant burden of cardiovascular disease. Older adults, in general, also have a greater benefit from metformin therapy than found in the TODAY trial. Thus, therapeutic approaches in older cohorts may not be optimal for youth with type 2 diabetes.

Intensive glucose control needs to be balanced against the potential for greater weight gain with insulin use and promotion of adipogenesis (33). Although insulin has anti-inflammatory systemic effects, excess weight gain may worsen insulin resistance and interfere with the beneficial effect of insulin and potentially worsen diabetic dyslipidemia. In support of this hypothesis, excessive weight gain (>4% of body weight) 6 months after the start of insulin therapy in adults with type 2 diabetes (the majority of whom were prescribed an oral glucose-lowering agent [metformin and/or sulfonylurea]) was associated with increased pro-inflammatory cytokine production in subcutaneous adipose tissue (34). Our prior publication (11) has indicated that insulin sensitivity was similar in those participants who progressed to insulin use as compared with those who did not. Although adolescents may gain weight and become more insulin resistant during puberty, the majority of subjects were post-pubertal (Table I). The TODAY cohort was obese, with a mean BMI of 34.9 ± 7.6 kg/m² at baseline (4). BMI did not change by 'time 0' (Table I) and remained stable over time. The effect of percent overweight on lipids was minimal. Nevertheless, we did find percent overweight to be a significant covariate in the results of inflammatory markers along with HbA1c and may thus interfere with the positive effect of insulin on inflammation. Interventions that simultaneously lower both glycemia and hyperinsulinemia and treatments to better target obesity may be more beneficial.

Limitations to the current analysis include variable follow-up over time. Insulin therapy was initiated following primary outcome with insulin glargine but then individualized with the goal of achieving glycemic control according to the treating physician's discretion based upon fasting glucose levels, rather than a target HbA1c. Although we have previously published results of adherence to oral diabetes medication prior to primary outcome (35), adherence to cardiovascular risk reduction therapies were not measured and total daily insulin dose cannot be verified. Medication and lifestyle adherence may explain in part the differences in our outcomes compared with adult trials that employed intensive insulin treatment for an extended duration.

It is disturbing that initiation of insulin therapy often slowed or halted but did not correct longitudinal adverse trends in the cardiovascular risk factors measured with the exception of PAI-1. Addition of insulin after failure of oral treatment regimen did not control the HbA1c; as such insulin has limited effects on lipids and inflammatory makers in the absence of glycemic control. Earlier initiation of insulin therapy, improved adherence, use of newer glycemic control agents, avoidance of excessive weight gain, novel approaches to increasing exercise and additional lipid lowering interventions need to be investigated in this high risk population.

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List of abbreviations:

apoB	Apolipoprotein B
BMI	Body mass index
HDL-C	High-density lipoprotein cholesterol
hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
LDL-C	Low-density lipoprotein cholesterol
LLM	Lipid lowering medications
M	Metformin monotherapy
M+L	Metformin plus intensive lifestyle intervention
M+R	Metformin plus rosiglitazone
NEFA	Plasma non-esterified fatty acids
PAI-1	Plasminogen activator inhibitor-1
TODAY	Treatment Options for type 2 Diabetes in Adolescents and Youth
VA CSDM	Veterans Affairs Cooperative Study in type II Diabetes Mellitus

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Figure I.

Plots of lipids showing slopes (solid line) before and after 'time 0' in the primary outcome group imposed on raw means. 'Time 0' is defined as the date long-term insulin therapy was started (vertical reference line). The short dashed line represents the raw means.

Figure II.

Plots of inflammatory markers showing slopes (solid line) before and after 'time 0' in the primary outcome group imposed on raw means. 'Time 0' is defined as the date long-term insulin therapy was started (vertical reference line). The short dashed line represents the raw means.

Figure III.

online Plots of LDL-C showing slopes before and after 'time 30' in the primary outcome group, by race-ethnicity (non-Hispanic Black: solid line; Hispanic: short dashed line; and non-Hispanic White: long dashed line). 'Time 0' is defined as the date long-term insulin therapy was started (vertical reference line).

Table IComparison of characteristics (mean $\pm$ SD or %) by analysis group at 'time 0'

	Never reached primary outcome group (n=363)	Reached primary outcome group (n=285)	p-value
Months from randomization to 'time 0'	18.7	19.0	ns
Characteristics and Outcomes			
Age (years)	15.4 $\pm$ 2.0	15.8 $\pm$ 2.4	ns
Female (%)	69.5%	63.4%	ns
Baseline Tanner stage (%)			
4-5	91.0%	86.8%	ns
<4	9.0%	13.2%	
Race-ethnicity (%)			
Non-Hispanic Black	26.5%	37.0%	0.0083
Hispanic	38.1%	39.9%	
Non-Hispanic White	27.3%	15.6%	
Other	8.1%	7.4%	
Parental history of dyslipidemia	27.3%	30.2%	ns
Parental history of cardiovascular disease	14.6%	17.0%	ns
BMI (kg/m ²)	35.5 $\pm$ 8.0	35.2 $\pm$ 7.6	ns
Percent overweight	74.4 $\pm$ 37.3	71.7 $\pm$ 36.7	ns
HbA1c (%)	5.8 $\pm$ 0.7	9.7 $\pm$ 1.7	<.0001
HbA1c (mmol/mol)	40 $\pm$ 8	83 $\pm$ 19	<.0001
Lipids			
On any lipid-lowering medications (% prescribed)	4.0%	8.6%	0.0430
Total cholesterol (mg/dL)	156.4 $\pm$ 33.5	166.1 $\pm$ 31.3	0.0013
LDL-C (mg/dL)	90.2 $\pm$ 27.3	95.0 $\pm$ 27.1	ns
HDL-C (mg/dL) - Females	43.5 $\pm$ 9.7	41.2 $\pm$ 9.1	0.0335
HDL-C (mg/dL) - Males	40.1 $\pm$ 7.8	38.7 $\pm$ 9.9	ns
Triglycerides (mg/dL) *	115.7 $\pm$ 75.1	149.1 $\pm$ 101.0	<.0001
ApoB (mg/dL)	80.0 $\pm$ 23.5	91.9 $\pm$ 22.6	<.0001
ApoB in LDL fractions (mg/dL)	51.2 $\pm$ 16.5	58.3 $\pm$ 16.2	<.0001
Cholesterol in LDL fractions (mg/dL)	80.3 $\pm$ 24.7	86.2 $\pm$ 24.2	0.0102
Ratio of apoB to cholesterol in LDL fractions	1.57 $\pm$ 0.12	1.49 $\pm$ 0.13	<.0001
Small LDL particle density (Rf <0.263)	46.8%	75.9%	<.0001
NEFA (plasma non-esterified fatty acids, mEq/L)	0.52 $\pm$ 0.20	0.62 $\pm$ 0.21	<.0001
Inflammatory markers			
PAI-1 (ng/mL) *	19.6 $\pm$ 14.6	27.0 $\pm$ 18.1	<.0001
hs-CRP (mg/dL) *	0.32 $\pm$ 0.45	0.48 $\pm$ 0.51	<.0001
Homocysteine (pmol/L) *	6.8 $\pm$ 1.9	6.7 $\pm$ 1.8	ns
IL-6 (pg/mL) *	2.0 $\pm$ 1.3	2.2 $\pm$ 1.3	0.0125

Medians are reported and compared using the Wilcoxon two-sample test for months from randomization to 'time 0' was defined as the mid-point in the study. Biological parental history of dyslipidemia (high cholesterol/high fat) and cardiovascular disease (stroke/heart attack) were obtained from the parent or other knowledgeable family member via self-report at the baseline visit. Laboratory values could not be obtained within 3 months of 'time 0' for all participants resulting in 15% missing laboratory values for the reached primary outcome group and 30% missing values for the never reached primary outcome group.

* Statistics were calculated on the original scale, p- values were based on tests performed on log transformed values to normalize the distribution.

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Table II

Tests of slopes before and after, 'time 0', for the primary outcome group in unadjusted and adjusted models

Outcomes	Unadjusted Models				Adjusted Models			
	slopes		p-values		slopes		p-values	
	before	after	before	after	before	after	before	after
Lipids								
Total cholesterol (mg/dL)	0.63	0.20	<.0001	<.0001	<.0001	<.0001	0.18	0.0019
LDL-C (mg/dL)	0.35	0.11	<.0001	0.0110	0.0006	0.09	0.06	0.0383
HDL-C (mg/dL)	0.065	0.009	<.0001	ns	0.0279	0.05	0.004	0.0073
Females								
Triglycerides (mg/dL)	0.010	0.024	ns	ns	ns	0.02	0.006	ns
Males								
Triglycerides (mg/dL) *	1.13	0.17	<.0001	ns	<.0001	0.39	0.04	0.0015
ApoB (mg/dL)	0.55	0.14	<.0001	<.0001	<.0001	0.18	0.08	<.0001
ApoB in LDL fractions (mg/dL)	0.24	0.05	<.0001	ns	<.0001	0.03	0.002	ns
Cholesterol in LDL fractions (mg/dL)	0.36	0.10	<.0001	0.0115	0.0002	0.13	0.07	0.0037
Ratio of apoB to cholesterol in LDL fractions	-0.0003	0.0010	ns	<.0001	0.0011	0.0011	0.0014	0.0007
NEFA (plasma non-esterified fatty acids, mEq/L)	0.0014	-0.0006	0.0003	ns	0.0045	-0.0002	ns	0.0201
Inflammatory markers								
PAI-1 (ng/mL) *	0.16	-0.09	<.0001	0.0007	<.0001	0.11	-0.06	0.0146
hs-CRP (mg/dL) *	0.0043	0.0037	<.0001	0.0001	0.0258	0.0035	0.0045	<.0001
Homocysteine (μmol/L) *	0.018	0.011	<.0001	0.0015	ns	0.022	0.012	<.0001
IL-6 (pg/mL) *	0.0069	0.0059	0.0266	0.0030	ns	0.0083	0.0097	<.0001

Data presented include trends over time (slopes) before and after the start of permanent insulin therapy at 'time 0'. These data are illustrated in Figures I and II. The three tests are (1) whether there is a significant slope before 'time 0', (2) whether there is a significant slope after 'time 0', and (3) whether there is a significant difference in slopes before vs. after 'time 0'. Adjusted models for the lipids were adjusted for HbA1c as a time-varying covariate only. Adjusted models for NEFA and inflammatory markers were adjusted for HbA1c and percent overweight.

* P-values were based on tests of log transformed variables to normalize the distribution; slopes were calculated on the original scale.

Table III

Tests of slopes before and after 'time 0' for the primary outcome group in unadjusted models, for participants who achieved a 1% reduction in HbA1c after 6 months on permanent insulin vs those who did not

Outcomes	Group who <u>reduced</u> their HbA1c by 1% after ~6 months on insulin				Group who <u>did not reduce</u> their HbA1c by 1% after ~6 months on insulin			
	slopes		p-values		slopes		p-values	
	before	after	before	after	before	after	before	after
Lipids								
Total cholesterol (mg/dL)	0.68	0.0002	<.0001	ns	0.0089	0.28	<.0001	0.0002
LDL-C (mg/dL)	0.42	0.04	<.0001	ns	0.0364	0.19	<.0001	0.0066
HDL-C (mg/dL)	0.012	0.046	ns	ns	ns	-0.005	0.0343	ns
	0.342	-0.125	ns	ns	0.0284	0.041	ns	ns
	1.03	-0.92	<.0001	0.0256	0.0004	0.43	<.0001	ns
Triglycerides (mg/dL) *	0.59	-0.03	<.0001	ns	0.0009	0.22	<.0001	0.0018
ApoB (mg/dL)	0.27	-0.03	<.0001	ns	0.0128	0.07	<.0001	ns
ApoB in LDL fractions (mg/dL)	0.38	0.02	0.0003	ns	ns	0.15	<.0001	0.0179
Cholesterol in LDL fractions (mg/dL)	-0.0008	0.0014	ns	0.0022	0.0150	0.0009	ns	0.0093
Ratio of apoB to cholesterol in LDL fractions								
NEFA (plasma non-esterified fatty acids, mEq/L)	0.0021	-0.0020	0.0306	ns	0.0470	-0.0001	ns	ns
Inflammatory markers								
PAI-1 (ng/mL) *	0.27	-0.08	0.0107	ns	0.0183	-0.12	0.0013	ns
Hs-CRP (mg/dL) *	-0.0005	0.0058	0.0266	ns	ns	0.003	<.0001	0.0272
Homocysteine (μmol/L) *	0.021	0.004	ns	ns	ns	0.010	0.0054	0.0403
IL-6 (pg/mL) *	0.0059	0.0065	ns	ns	ns	0.0111	ns	0.0022

The three tests are (1) whether there is a significant slope before 'time 0', (2) whether there is a significant slope after 'time 0', and (3) whether there is a significant difference in slopes before vs. after 'time 0'.

* P-values were based on tests of log transformed variables to normalize the distribution; slopes were calculated on the original scale.

Table IV

Tests of LDL-C slopes before and after, time 0^{*} for the primary outcome group in unadjusted and adjusted models, by race/ethnicity

Outcomes	Unadjusted Models				Adjusted Models			
	slopes		p-values		slopes		p-values	
	before	after	before	after	before	after	before	after
LDL-C (mg/dL)								
Non-Hispanic Black	0.39	0.11	<.0001	ns	0.02	0.01	-0.03	ns
Hispanic	0.45 [*]	0.03	<.0001	ns	0.0001	0.24	-0.01	0.0002
Non-Hispanic White	0.13 [*]	0.16	0.048	ns	ns	0.01	0.13	ns

Data presented include trends over time (slopes) before and after the start of permanent insulin therapy at time 0 for each race- ethnicity group. The three tests are (1) whether there is a significant slope before, time 0^{*}, (2) whether there is a significant slope after, time 0^{*}, and (3) whether there is a significant difference in slopes before vs. after, time 0^{*}. Adjusted models were adjusted for HbA1c as a time-varying covariate.

A significant difference between Hispanic and Non-Hispanic White slopes for LDL-C was found before insulin start in unadjusted models (p=0.0065; see Figure III online).