Published in final edited form as:

Pediatr Diabetes. 2016 June ; 17(4): 257–265. doi:10.1111/pedi.12277.

Lipoprotein Subfraction Cholesterol Distribution is More Atherogenic in Insulin Resistant Adolescents with Type 1 Diabetes

Melanie Cree-Green^{1,2,3}, David M. Maahs¹, Annie Ferland⁴, John E. Hokanson⁴, Hong Wang⁴, Laura Pyle², Gregory L. Kinney^{2,5}, Martina King⁴, Robert H. Eckel⁴, and Kristen J. Nadeau^{1,2,3}

¹Barbara Davis Center for Childhood Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO

²Department of Pediatrics, Division of Pediatric Endocrinology, University of Colorado Anschutz Medical Campus, Aurora, CO

³Center for Women's Health Research, Aurora, CO

⁴Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO

⁵Department of Epidemiology, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO

Abstract

Aims/hypothesis—Adolescents with type 1 diabetes (T1D) often have a less atherogenic-appearing fasting lipid profile than controls, despite increased rates of cardiovascular disease (CVD) as adults. We previously reported an atherogenic lipoprotein subfraction cholesterol distribution associated with insulin resistance (IR) in T1D adults. We sought to determine if T1D youth have more atherogenic profile than controls via a cross-sectional study.

Methods—Following 3 days of controlled diet and restricted exercise, fasting plasma samples were drawn from 28 T1D youth (50% female, age 15.3±2 years, BMI 48%ile; diabetes duration 73 ± 52 months, HbA1c 8.3±1.4%) and 17 non-diabetic controls (47% female, age 15.0±2 years, BMI 49%ile) prior to a hyperinsulinemic euglycemic clamp. Lipoproteins were fractionated by fast protein liquid chromatography (FPLC) and lipoprotein cholesterol distribution determined. Outcome measures were IR assessed by glucose infusion rate (GIR) and FPLC lipoprotein subfraction cholesterol distribution.

Address all correspondence to: Kristen J. Nadeau, MD, MS, Pediatric Endocrinology, Children's Hospital Colorado, Box 265, 13123 E 16th Ave, Aurora, CO 80045, Phone: 720 777-6128, Fax: 720 777-7301, ; Email: kristen.nadeau@childrenscolorado.org

Author Contributions:

M.C.G researched data and wrote the manuscript. D.M.M researched data and wrote the manuscript. A.F researched data and edited the manuscript, J.E.H. researched data and edited the manuscript, H.W. researched data and edited the manuscript, G.L. K. performed statistical analysis and edited the manuscript, L.X.P. performed statistical analysis and edited the manuscript, M.K. researched data and edited the manuscript, R.H.E. researched data and edited the manuscript, K.J.N researched data, contributed to discussion and edited the manuscript.

Results—T1D youth were more IR (GIR 9.1 ± 3.6 vs. 14.7 ± 3.9 mg/kg/min, $p < 0.0001$) and had more cholesterol distributed as small dense LDL-C and less as large buoyant HDL-C than controls ($p < 0.05$), despite no differences in the fasting lipid panel. T1D girls lacked the typical female less-atherogenic profile, whereas control girls tended to have a shift towards less dense LDL-C and HDL-C vs. control boys. Among T1D, IR but not HbA1c was associated with a more atherogenic lipoprotein profile.

Conclusions/Interpretations—Normal weight T1D youth, especially females, had more atherogenic LDL-C and HDL-C distributions which correlated with lower insulin sensitivity. IR may contribute to the increased CVD burden in T1D.

Keywords

Type 1 Diabetes; Cardiovascular disease; Lipids; Insulin resistance; pediatrics

Introduction

Adults with type 1 diabetes (T1D) have a less atherogenic fasting lipid profile (higher high density lipoprotein cholesterol, HDL-C; lower low density lipoprotein cholesterol, LDL-C and triglycerides, TG) than people without diabetes [^{1, 2}], but paradoxically have increased rates of cardiovascular disease (CVD)[^{3–5}]. The typical protective effect of female sex on CVD risk factors is also lost in women with T1D, who therefore develop CVD earlier than non-diabetic women and have CVD rates approaching those of men with T1D [^{6–9}].

Alterations in the lipoprotein subfraction cholesterol distribution, including increased concentrations of small LDL particles, offer markers of CVD risk beyond the standard fasting lipid profile. Previously, we investigated differences in lipoprotein subfraction cholesterol distribution between adults with and without T1D, by sex, and how insulin resistance (IR) affects this distribution [¹⁰]. Women with T1D had more LDL-C and a shift to a smaller LDL size. Moreover, in men and women with T1D, IR, but not HbA1C, was associated with a more atherogenic lipoprotein profile. These data suggest that differences in lipoprotein cholesterol distribution may contribute to CVD risk in T1D. Further, IR may mediate increased CVD risk in adults with T1D via changes in lipoprotein cholesterol distribution. Recently, changes in lipoprotein subparticles were found after statin therapy in youth with T1D indicating that particle size may be useful as a therapeutic marker [¹¹].

Detailed lipoprotein profiles have not been performed in youth with T1D, and thus it is unclear when these abnormal patterns develop, and what modifiable variables are associated with the development of unfavorable profiles. If such pathophysiologic lipoprotein differences exist in adolescence, this could argue for early intervention. Adolescents with T1D and a HbA1c below target ($< 7.5\%$) also were reported to have similar (total cholesterol, TC and LDL-C) or even less atherogenic (TG, HDL-C, and non-HDL-C) standard lipids than controls, whereas those with A1c above target had elevated TC, LDL-C, and non-HDL-C, but normal TG and HDL-C [¹²]. We have also shown previously that adolescents with T1D are more IR than non-diabetic control adolescents of the same BMI, and increases in IR were not related to glycemic control as assessed with HbA1C [¹³]. Further estimated IR in a large cohort of youth with T1D was associated with increased LDL-C [¹⁴]. However, it is

unknown whether IR impacts lipoprotein distribution as early as adolescence. Therefore, in this study we aimed to extend our findings in adults with T1D into adolescence, testing the hypothesis that the pro-atherogenic derangements in lipoprotein subfraction cholesterol distribution we reported in T1D adults are already present early in the course of T1D and are modified by glycemia and IR^[10].

Research and Design Methods

Study population

45 participants 12–18 years of age were recruited from pediatric clinics at the Children's Hospital Colorado and the Barbara Davis Center for Childhood Diabetes, both academic tertiary care referral centers, between 2009–2013 for a 2 visit cross-sectional study. Participants included non-obese (BMI 95th percentile for age) youth with and without T1D. Screening included a history, physical examination, Tanner staging, activity assessment and fasting laboratory testing as previously described, with a preliminary phone screening so that all subjects who signed the consent and participated in the screening visit were studied^[15]. All participants were untrained (defined as less than 3 hours per week of exercise, verified by a standardized 3-day activity recall, and a 7-day accelerometer, Actigraph, Pensacola, FL), and had achieved Tanner Stage 2 or above in puberty (as assessed by physical exam by a pediatric endocrinologist). T1D was defined by American Diabetes Association (ADA) criteria, plus the presence of at least one antibody associated with autoimmune diabetes (glutamic acid decarboxylase (GAD), islet cell (ICA-2) or insulin (IAA) autoantibodies) as well as insulin requirement. In non-diabetic participants, diabetes was ruled out by a 2-hour oral glucose tolerance test and HbA1c. Exclusions included resting BP >140/90 mmHg, hemoglobin <9mg/dl, serum creatinine >1.5 mg/dl, smoking, medications affecting IR (oral or inhaled steroids, metformin, thiazolidinediones, atypical antipsychotics), anti-diabetic drugs other than insulin, antihypertensive medications, statins, pregnancy, breastfeeding, plans to alter exercise or diet during the study, and for T1D participants, HbA1c >12%. Subjects with T1D had a negative 1st degree family history for type 2 diabetes. This study was approved by the University of Colorado Anschutz Medical Campus Institutional Review Board. Parental informed consent and participant assent was obtained from all participants less than 18 years old and participant consent from those 18 years or older.

Hyperinsulinemic-Euglycemic clamp

The study day was preceded by 3 days of restricted physical activity and a fixed-macronutrient, weight-maintenance diet (55% carbohydrates, 30% fat, 15% protein). A hyperinsulinemic-euglycemic clamp (80 mU/m²/min of insulin) was performed in the AM after an observed overnight fast to estimate insulin sensitivity as previously described^[13]. Glucose infusion rate, GIR (mg/kg/min) was measured based on steady-state measurements from the final 30 min of the clamp.

T1D participants were instructed to replace any long-acting insulin injections in the 24 hours prior to admission with short-acting insulin, so that no long-acting insulin was given within 36 hours of the clamp baseline blood draw. Subcutaneous insulin was discontinued 2 hours

after a 6 PM dinner meal bolus and participants were then maintained overnight on intravenous regular insulin with adjustments by a standard protocol to maintain near euglycemia (goal blood sugar 100 mg/dl) until starting the clamp the next morning [13]. For all participants, fasting blood was collected for laboratory analyses at the University of Colorado Clinical-Translational Research Center Laboratory.

Measurement of Lipid Profiles

Fasting plasma samples were obtained prior to initiation of the clamp for measurement of TC, HDL-C, and TG. Measurements of TC, HDL-C, and TG were performed enzymatically on a Hitachi 917 autoanalyzer (Boehringer Mannheim Diagnostics, Indianapolis, Ind). LDL-C levels were calculated by the Friedewald equation. 1.

Other Variables

Weight and height were measured using standard methods and body mass index (BMI), defined as weight (kg) divided by height (meters squared), was calculated. Minimal waist circumference was measured. HbA1c levels were measured by (DCCT-calibrated) ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, Calif). Body composition by DEXA was performed by standard methods as previously described to determine fat free mass [13].

Lipoprotein Analysis

Individual participant's fasting EDTA plasma samples (250µL) were chromatographed via fast protein liquid chromatography (FPLC) using two Superose 6 columns in series as previously reported[10]. Fifty-one 0.5 mL fractions were collected. Cholesterol was measured in each fraction using a commercially available kit (Cayman Chemical Company) following procedures outlined in the package insert.

Statistical Analyses

FPLC was a secondary outcome and the primary outcome was the difference in GIR between participants with T1D and controls. A minimum of 15 participants in each group was needed to detect a clinically significant difference via 2 tailed T test assuming a power of 80% and an alpha 0.05. Variables were examined for normality, and non-normally distributed variables were log transformed for analysis. Differences in clinical and clamp parameters between T1D and non-DM participants and between males and females within each group were examined using unpaired Student's *t* tests. Differences in categorical variables were examined using chi-square tests. A p-value of <0.05 was considered statistically significant. SAS version 9.3 (Cary, NC) was used for analyses and Sigma Plot 11.2 Systat Software, Inc. San Jose, CA) was used for generating difference figures.

Methods for Presentation of Figures

The cholesterol content in each fraction obtained from FPLC was expressed as the percent of the total cholesterol in all sub-fractions to adjust for differences in total cholesterol levels between participants, adapting the methodology previously used in DCCT/EDIC trials[16]. This percent was calculated by summing the cholesterol for an individual in all 51 fractions

and expressing the result for each fraction as the cholesterol in that fraction divided by the summed cholesterol and multiplied by 100. To test the significance of differences in cholesterol distributions between groups of participants, a difference plot was generated by subtracting the mean percent cholesterol value of each fraction in one group from the mean percent cholesterol value in the same fraction of the second group and determining the 95% confidence interval (CI) for this difference. *A difference in fractional cholesterol content between groups is significant ($P<0.05$) when the 95% CI does not cross the zero line.*

Results

Characteristics of the 45 study participants are presented in Table 1 stratified by gender and T1D status. Participants were similar for age, race-ethnicity, Tanner stage, BMI, % lean mass and % fat mass. As expected, male and female T1D participants had higher HbA1c and lower insulin sensitivity than controls (mean T1D GIR 9.1 ± 3.6 vs. mean control GIR 14.7 ± 3.9 mg/kg/min, $p<0.0001$). Mean fasting blood sugar for controls was 85 ± 2 mg/dL, and for T1D 133 ± 6 , $p<0.001$. However, glucose concentrations at the end of the clamp were identical (Control 100 ± 2 , T1D 100 ± 2).

Lipid differences by type 1 diabetes

Table 1 shows the fasting lipid profile, which shows no differences between T1D or control participants. The mean lipoprotein subfraction cholesterol distributions are displayed within participants with T1D and within non-diabetic participants by gender (Figure 1A) and group differences in Figure 1B. Participants with T1D had multiple LDL fractions that were significantly higher than controls, and multiple HDL fractions that were significantly lower than non-diabetic controls ($p<0.05$ for both). In addition, T1D adolescents had a shift towards increased cholesterol in denser LDL and HDL.

Differences by sex

Differences by sex in lipoprotein subfraction cholesterol distribution are displayed within participants with T1D (Figure 2A) and within non-diabetic controls (Figure 2B). There were no notable differences between males with T1D and females with T1D, while non-diabetic males had a non-significant shift towards increased cholesterol in denser LDL and HDL compared to non-diabetic females. We next investigated differences in lipoprotein subfraction cholesterol distribution by T1D status in females (Figure 2C) and males (Figure 2D). Among T1D females, cholesterol in VLDL was lower in several fractions, and in both males and females, those with T1D had multiple LDL fractions with significantly more cholesterol and multiple HDL fractions with significantly less cholesterol than non-diabetic control participants, as highlighted by the arrows in Figures 2C and 2D.

Relationship between FPLC and glucose control or insulin sensitivity

To investigate the influence of hyperglycemia or insulin sensitivity on lipoprotein subfraction cholesterol distribution in youth with T1D, a sub-analysis was conducted in youth with T1D. Subjects were divided into two groups, with a cut-point of 8.0% for HbA1c. There were no significant lipoprotein subfraction cholesterol differences in subjects with an HbA1c above ($N=15$) or below 8% ($N=13$), as shown in Figure 3A. For insulin

sensitivity, FPLC data for those subjects whose GIR was either below or above the mean GIR for the group were compared, as shown in Figure 3B. Subjects with a GIR in the lower half, (i.e. lower insulin sensitivity), had more fractions of cholesterol distributed as very low density lipoprotein (VLDL) and three less fractions as HDL with a shift towards more cholesterol in dense HDL.

Discussion

The risk of CVD is increased in individuals with T1D, in particular women, despite an overall less atherogenic standard lipid profile than non-diabetic individuals [6]. However, we previously reported that adults with T1D had a more atherogenic lipoprotein subfraction profile by FPLC [17]. We now demonstrate that these alterations in lipoprotein subfraction cholesterol profile are already present in youth with T1D, indicating that alterations in lipid composition occur within several years of T1D diagnosis in youth. Specifically, we found that both male and female adolescents with T1D had a larger proportion of cholesterol distributed in dense LDL and less distributed in HDL-C, compared to non-diabetic youth of similar age, Tanner Stage and BMI, despite no differences in the standard fasting lipid panel. Moreover, girls with T1D lacked some of the typical gender-protective effect seen in girls without diabetes, as previously described in adult women with T1D. Therefore, these findings suggest that the standard fasting lipid profile may inadequately assess lipoprotein health and CVD risk in people with T1D and this is the first time this has been demonstrated in youth.

We also found that directly measured IR was associated with a pro-atherogenic lipoprotein profile in youth with T1D which has not previously been explored in youth. These findings are consistent with our results from adults with T1D, perhaps indicating that lipid abnormalities develop early in the pathophysiology of diabetes, which would relate to other early cardiovascular abnormalities seen in youth [18–20]. The relationship between IR and serum lipids is well established in adults without diabetes. In adults with IR but not T1D, IR was not associated with the total serum concentration of LDL-C, but did relate to LDL particle size and fraction profile [21]. Further, 41% of LDL particle size was related to IR as determined by regression analysis [21]. In a cohort of 304 adults with T1D, IR was closely associated with increases in total cholesterol and LDL-C [22]. We previously found that estimated IR is associated with increased CVD risk factors in youth with T1D (blood pressure, LDL-C, high sensitivity C-reactive protein, and body mass index z score) [14]. Furthermore, a meta-analysis of 9 metformin studies in adults with T1D showed improved lipid profile, and lower daily insulin needs as a surrogate for improved insulin sensitivity [23]. We also found that low-dose metformin therapy in youth with T1D decreased total daily insulin dose, but further studies are needed to determine if improving insulin sensitivity in T1D would also improve lipoprotein profiles in youth [24]. Finally, progression through puberty has been shown to influence IR, with peak IR found at Tanner stage 3 [25]. As our groups are similar for Tanner stage it is unlikely that pubertal progression is influencing the difference between the groups. Thus, the relationship between IR and altered serum lipids is consistent in patients with T1D, and treatment of IR may be an important additional therapeutic option to be initiated in youth.

It is not clear if hepatic production or serum metabolism and clearance of lipoproteins is altered in T1D. The liver plays a large role in lipoprotein processing, and hepatic IR may be related to alterations in the lipoprotein profile. Animal models of primary hepatic IR demonstrate its causal role in the development of non-alcoholic fatty liver disease, type 2 diabetes and CVD [17, 26]. Peripheral delivery of insulin, characteristic of T1D, bypasses portal delivery of insulin to the liver and allows for persistence of glucagon secretion, decreases IGF-1 secretion, and in turn, through reduced negative feedback, increases growth hormone secretion. The increased glucagon and growth hormone prevent the liver from fully switching into a fed state in terms of suppression of hepatic glucose release and contribute to hyperglycemia [27, 28] and IR. However, neither fasting nor post-prandial rates of lipogenesis are altered in adults with T1D when compared to healthy controls [29]. Moreover, lipid transport studies in men with T1D demonstrated normal LDL-C plasma clearance, cholesterol esterification and lipid transfer to HDL-C [30]. However, when insulin is injected subcutaneously vs. intraperitoneally in patients with well controlled T1D more cholesteryl ester transfer from HDL-C to apolipoprotein B containing particles has been demonstrated [31]. Moreover, increases in plasma cholesteryl ester exchange from HDL-C to pro-atherogenic lipoproteins has been demonstrated in insulin-resistant states [32, 33]; and also in LDL size [34]. Therefore, subcutaneous delivery of insulin may negatively impact glucose and lipid metabolism.

Diet may also play a role in alteration of the lipoprotein profile in youth with T1D. High fat or high simple carbohydrate diets, as well as failure to use carbohydrate counting are all associated with dyslipidemia in youth with T1D [35–38]. In our study, we did control for the acute effects of diet by providing a three-day weight maintenance diet with a fixed percentage of macronutrients prior to testing. Thus, our results are not reflective of an acute difference in dietary intake between groups.

The role of blood sugar control in influencing serum lipid profiles is not clear. Subtle differences in total lipid profiles and HbA1C have been found in large cohort studies including many subjects not near goal HbA1C [12, 18] [39] [40]. Pre-DCCT studies also indicate that HbA1c levels even higher than our upper cut-off may be associated with altered lipid profiles [9]. However, when only subjects near goal HbA1C were studied, carotid intimal medial thickness, a marker of atherosclerotic plaque development, was increased in T1D youth, but did not relate to HbA1C [41]. Further, glycemic variability, but not HbA1c, related to coronary artery calcification in men with T1D [42]. Moreover, we found no relationship between HbA1c and alterations in the lipoprotein profile in the HbA1c range of 6.5–<12%, although our sample size may be underpowered to detect this association. We specifically allowed for a wider range of HbA1C, as this range is typical for youth, as indeed less than 50% of adolescents are meeting recommended HbA1C goals [43]. Thus, it appears that blood sugar concentrations may influence lipids when the blood sugars are excessively high, but at moderate to slightly poor control typically seen in youth today, the effect of blood sugar on serum lipids may be less important.

Females with T1D paradoxically appear to have more proatherogenic lipid profiles than males. We performed identical analysis methods to those utilized in a large cohort of adults with T1D, enrolled as part of the CACTI study. Our findings in adolescents with T1D are

similar to those in CACTI adults, namely a worse lipoprotein profile in T1D youth relative to controls [10]. Specifically, both female youth and adults with T1D have more dense LDL compared to controls, and youth with T1D also had lower HDL. Similar to our pediatric data, IR, but not glycemia, was also associated with a worse lipoprotein profile in CACTI adults, particularly in women. Increased CVD risk markers such as elevated LDL was also observed in women enrolled in the DCCT [4]. When serum from these women was fractionated by nuclear magnetic resonance (NMR), they had more cholesterol distributed in the middle density of LDL [9]. Colhoun et al also found that women with T1D had more pro-atherogenic lipid profiles, with more cholesterol distributed in small LDL subfractions [44]. When combining the results of these studies with our current findings, the risk seen in females with T1D appears to be consistent across the lifespan, with IR being a likely underlying cause. However, our pediatric gender differences were not as pronounced as those found in adults, likely because our study included some participants who had not completed puberty, and thus have lower levels of sex steroids and less sexual dimorphic characteristics.

The relationship between HDL-C, LDL-C, and LDL size and CVD has been demonstrated in youth and adults with T1D. Increased serum concentrations of small LDL were shown to be associated with multiple CVD markers, including hypertension, larger waist/hip ratio and lower HDL-C concentration [45]. HDL-C and BMI were found to be the primary determinants of carotid intimal medial thickness (CIMT) in young adults with T1D [46]. Additionally, weight gain over time was related to both decreases in HDL-C and increases in CIMT in adults with T1D followed in the DCCT [47]. While we did not examine the role of BMI in this study, which focused on normal weight participants, the pediatric obesity epidemic will likely worsen the reported IR and lipoprotein abnormalities. In a large longitudinal study in youth with T1D, pulse wave velocity as a marker of arterial stiffness was also associated with increases in waist circumference and LDL-C [48]. Moreover, ATP guidelines have consistently called for lower LDL-C and total cholesterol concentrations in adults with T1D [49, 50]. Our findings extend this knowledge by suggesting that a more detailed lipid profile in patients with T1D may improve CVD risk stratification. Further, formal guidelines and early intervention for youth with T1D are also needed.

In conclusion, our results add to a growing body of evidence that the standard clinical lipid profile may not detect early abnormalities in patients with T1D. Moreover, we found that non-obese T1D youth, particularly females, have a more atherogenic lipoprotein profile which is related to IR and likely contributes to the early development of CVD in T1D. Future studies should examine whether improvement in IR can alter the lipoprotein profile, to reduce CVD in this patient population.

Acknowledgments

The authors would like to thank the participants and their families for participating.

Funding:

K.J.N.: NIH NCRR K23 RR020038-01, NIH/NCRR Colorado CTSI Co-Pilot Grant TL1 RR025778, NIH/NIDDK IR56DK088971-01, Juvenile Diabetes research Foundation 5—2008-291, American Diabetes Association 7-11-CD-08.

M.C.G.: Pediatric Endocrinology Fellowship training grant NIDDK T32 DK063687, O'Brien fellowship in Diabetes, Pediatric Endocrine Society Fellowship, NIH BIRCWH 2K12HD057022.

UNIVERSITY: Adult GCRC NIH Grant #M01-RR00051; Pediatric CTRC NIH Grant #5MO1 RR00069.

Abbreviations

ADA	American Diabetes Association
CI	confidence interval
CVD	cardiovascular disease
GIR	Glucose infusion rate
GAD	glutamic acid decarboxylase
FPLC	fast protein liquid chromatography
IAA	insulin autoantibodies
IR	insulin resistance
ICA-2	islet cell
T1D	type 1 diabetes
TG	triglycerides
VLDL	very low density lipoprotein VLDL

References

1. Maahs DM, Maniatis AK, Nadeau K, Wadwa RP, McFann K, Klingensmith GJ. Total cholesterol and high-density lipoprotein levels in pediatric subjects with type 1 diabetes mellitus. *J Pediatr*. 2005; 147:544–546. [PubMed: 16227045]
2. Wadwa RP, Kinney GL, Maahs DM, et al. Awareness and treatment of dyslipidemia in young adults with type 1 diabetes. *Diabetes Care*. 2005; 28:1051–1056. [PubMed: 15855566]
3. Maahs DM, Wadwa RP, Bishop F, Daniels SR, Rewers M, Klingensmith GJ. Dyslipidemia in youth with diabetes: to treat or not to treat? *J Pediatr*. 2008; 153:458–465. [PubMed: 18847618]
4. Group DR. Lipid and lipoprotein levels in patients with IDDM diabetes control and complication. Trial experience. *Diabetes Care*. 1992; 15:886–894. [PubMed: 1516509]
5. Lachin JM, Orchard TJ, Nathan DM. Update on cardiovascular outcomes at 30 years of the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care*. 2014; 37:39–43. [PubMed: 24356596]
6. Laing SP, Swerdlow AJ, Slater SD, et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia*. 2003; 46:760–765. [PubMed: 12774166]
7. Colhoun HM, Rubens MB, Underwood SR, Fuller JH. The effect of type 1 diabetes mellitus on the gender difference in coronary artery calcification. *J Am Coll Cardiol*. 2000; 36:2160–2167. [PubMed: 11127456]
8. Lloyd CE, Kuller LH, Ellis D, Becker DJ, Wing RR, Orchard TJ. Coronary artery disease in IDDM. Gender differences in risk factors but not risk. *Arterioscler Thromb Vasc Biol*. 1996; 16:720–726. [PubMed: 8640398]
9. Jenkins AJ, Lyons TJ, Zheng D, et al. Serum lipoproteins in the diabetes control and complications trial/epidemiology of diabetes intervention and complications cohort: associations with gender and glycemia. *Diabetes Care*. 2003; 26:810–818. [PubMed: 12610042]

10. Maahs DM, Hokanson JE, Wang H, et al. Lipoprotein subfraction cholesterol distribution is proatherogenic in women with type 1 diabetes and insulin resistance. *Diabetes*. 2010; 59:1771–1779. [PubMed: 20393149]
11. Canas JA, Ross JL, Taboada MV, et al. A randomized, double blind, placebo-controlled pilot trial of the safety and efficacy of atorvastatin in children with elevated low-density lipoprotein cholesterol (LDL-C) and type 1 diabetes. *Pediatr Diabetes*. 2014
12. Guy J, Ogden L, Wadwa RP, et al. Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. *Diabetes Care*. 2009; 32:416–420. [PubMed: 19092167]
13. Nadeau KJ, Regensteiner JG, Bauer TA, et al. Insulin Resistance in Adolescents with Type 1 Diabetes and Its Relationship to Cardiovascular Function. *Journal of Clinical Endocrinology & Metabolism*. 2010; 95:513–521. [PubMed: 19915016]
14. Specht BJ, Wadwa RP, Snell-Bergeon JK, Nadeau KJ, Bishop FK, Maahs DM. Estimated insulin sensitivity and cardiovascular disease risk factors in adolescents with and without type 1 diabetes. *J Pediatr*. 2013; 162:297–301. [PubMed: 22921593]
15. Maahs DM, Nadeau K, Snell-Bergeon JK, et al. Association of insulin sensitivity to lipids across the lifespan in people with Type 1 diabetes. *Diabet Med*. 2011; 28:148–155. [PubMed: 21219421]
16. Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. *Diabetes Control and Complications Trial*. *Jama*. 1998; 280:140–146. [PubMed: 9669786]
17. Biddinger SB, Hernandez-Ono A, Rask-Madsen C, et al. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab*. 2008; 7:125–134. [PubMed: 18249172]
18. Kuryan RE, Jacobson MS, Frank GR. Non-HDL-cholesterol in an adolescent diabetes population. *Journal of clinical lipidology*. 2014; 8:194–198. [PubMed: 24636179]
19. Alman AC, Talton JW, Wadwa RP, et al. Cardiovascular health in adolescents with type 1 diabetes: The SEARCH CVD Study. *Pediatr Diabetes*. 2014
20. Donaghue KC, Wadwa RP, Dimeglio LA, et al. Microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes*. 2014; 15(Suppl 20):257–269. [PubMed: 25182318]
21. Goff DC Jr, D'Agostino RB Jr, Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. *Metabolism*. 2005; 54:264–270. [PubMed: 15690322]
22. Bulum T, Duvnjak L. Insulin resistance in patients with type 1 diabetes: relationship with metabolic and inflammatory parameters. *Acta clinica Croatica*. 2013; 52:43–51. [PubMed: 23837272]
23. Vella S, Buetow L, Royle P, Livingstone S, Petrie JR. Metformin in type 1 diabetes reduces insulin requirements without significantly improving glycaemic control. Reply to Schatz H [letter]. *Diabetologia*. 2011; 54:203–204. [PubMed: 21046359]
24. Nadeau KJ, Chow K, Alam S, et al. Effects of low dose metformin in adolescents with type I diabetes mellitus: a randomized, double-blinded placebo-controlled study. *Pediatr Diabetes*. 2014
25. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. *Pediatric research*. 2006; 60:759–763. [PubMed: 17065576]
26. Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med*. 2005; 11:183–190. [PubMed: 15685173]
27. Thankamony A, Tossavainen PH, Sleight A, et al. Short-term administration of pegvisomant improves hepatic insulin sensitivity and reduces soleus muscle intramyocellular lipid content in young adults with type 1 diabetes. *J Clin Endocrinol Metab*. 2014; 99:639–647. [PubMed: 24423298]
28. Bergman BC, Howard D, Schauer IE, et al. Features of hepatic and skeletal muscle insulin resistance unique to type 1 diabetes. *J Clin Endocrinol Metab*. 2012; 97:1663–1672. [PubMed: 22362823]

29. Lambert JE, Ryan EA, Thomson AB, Clandinin MT. De novo lipogenesis and cholesterol synthesis in humans with long-standing type 1 diabetes are comparable to non-diabetic individuals. *PLoS ONE*. 2013; 8:e82530. [PubMed: 24376543]
30. Feitosa AC, Feitosa-Filho GS, Freitas FR, Wajchenberg BL, Maranhao RC. Lipoprotein metabolism in patients with type 1 diabetes under intensive insulin treatment. *Lipids in health and disease*. 2013; 12:15. [PubMed: 23398881]
31. Bagdade JD, Dunn FL, Eckel RH, Ritter MC. Intraperitoneal insulin therapy corrects abnormalities in cholesteryl ester transfer and lipoprotein lipase activities in insulin-dependent diabetes mellitus. *Arterioscler Thromb*. 1994; 14:1933–1939. [PubMed: 7981182]
32. Coniglio RI, Merono T, Montiel H, et al. HOMA-IR and non-HDL-C as predictors of high cholesteryl ester transfer protein activity in patients at risk for type 2 diabetes. *Clinical biochemistry*. 2012; 45:566–570. [PubMed: 22366373]
33. de Vries R, Borggreve SE, Dullaart RP. Role of lipases, lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in abnormal high density lipoprotein metabolism in insulin resistance and type 2 diabetes mellitus. *Clinical laboratory*. 2003; 49:601–613. [PubMed: 14651331]
34. Sandhofer A, Kaser S, Ritsch A, et al. Cholesteryl ester transfer protein in metabolic syndrome. *Obesity (Silver Spring)*. 2006; 14:812–818. [PubMed: 16855190]
35. Bortsov AV, Liese AD, Bell RA, et al. Sugar-sweetened and diet beverage consumption is associated with cardiovascular risk factor profile in youth with type 1 diabetes. *Acta diabetologica*. 2011; 48:275–282. [PubMed: 21249401]
36. Dalsgaard H, Saunders C, Padilha Pde C, Luescher JL, Szundy Berardo R, Accioly E. Glycemic control and lipid profile of children and adolescents undergoing two different dietetic treatments for type 1 diabetes mellitus. *Nutricion hospitalaria*. 2014; 29:547–552. [PubMed: 24558997]
37. The NS. Crandell JL, Thomas J, et al. Correlates of medical nutrition therapy and cardiovascular outcomes in youth with type 1 diabetes. *Journal of nutrition education and behavior*. 2013; 45:661–668. [PubMed: 23891147]
38. Meissner T, Wolf J, Kersting M, et al. Carbohydrate intake in relation to BMI, HbA1c and lipid profile in children and adolescents with type 1 diabetes. *Clin Nutr*. 2014; 33:75–78. [PubMed: 23642393]
39. Maahs DM, Ogden LG, Dabelea D, et al. Association of glycaemia with lipids in adults with type 1 diabetes: modification by dyslipidaemia medication. *Diabetologia*. 2010; 53:2518–2525. [PubMed: 20820753]
40. Maahs DM, Dabelea D, D'Agostino RB Jr. Glucose control predicts 2-year change in lipid profile in youth with type 1 diabetes. *J Pediatr*. 2013; 162:101–107. e101. [PubMed: 22795314]
41. El Samahy MH, Matter RM, Youssef OI, Shams El Din El Telbany MA, Kamal NA. Relation between carotid intima media thickness and oxidative stress markers in type 1 diabetic children and adolescents. *Journal of diabetes and metabolic disorders*. 2013; 12:50. [PubMed: 24355617]
42. Snell-Bergeon JK, Roman R, Rodbard D, et al. Glycaemic variability is associated with coronary artery calcium in men with Type 1 diabetes: the Coronary Artery Calcification in Type 1 Diabetes study. *Diabet Med*. 2010; 27:1436–1442. [PubMed: 21059097]
43. Wood JR, Miller KM, Maahs DM, et al. Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care*. 2013; 36:2035–2037. [PubMed: 23340893]
44. Colhoun HM, Otvos JD, Rubens MB, Taskinen MR, Underwood SR, Fuller JH. Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. *Diabetes*. 2002; 51:1949–1956. [PubMed: 12031985]
45. Erbey JR, Robbins D, Forrest KY, Orchard TJ. Low-density lipoprotein particle size and coronary artery disease in a childhood-onset type 1 diabetes population. *Metabolism*. 1999; 48:531–534. [PubMed: 10206450]
46. Pinto CS, Lana JM, Gabbay MA, de Sa JR, Dib SA. HDL cholesterol levels and weight are the main determinants of subclinical atherosclerosis in the young with type 1 diabetes and suitable glycaemic control. *Diab Vasc Dis Res*. 2014; 11:125–128. [PubMed: 24553254]

47. Purnell JQ, Zinman B, Brunzell JD. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) study. *Circulation*. 2013; 127:180–187. [PubMed: 23212717]
48. Dabelea D, Talton JW, D'Agostino R Jr. Cardiovascular risk factors are associated with increased arterial stiffness in youth with type 1 diabetes: the SEARCH CVD study. *Diabetes Care*. 2013; 36:3938–3943. [PubMed: 24101697]
49. Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013
50. Stone NJ, Robinson J, Lichtenstein AH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013

Figure 1. Lipoprotein Subfraction Data from Youth with and without T1D

A Overall plot of percent distribution of cholesterol in each of the study groups.

B Absolute differences in FPLC lipoprotein distribution by T1D status (mean proportion in T1D in each lipoprotein subfraction minus mean proportion in control in each lipoprotein subfraction, so that a mean above zero indicates more cholesterol in T1D participants and a mean below zero indicates less.”Arrows indicate fractions in which statistically significant differences exist.

Figure 2. Differences by Gender and Diabetes Status

- A. Differences in FPLC lipoprotein distribution by sex in T1D participants (Male T1D – Female T1D).
- B. Differences in FPLC lipoprotein distribution by sex in control participants (Male control – Female control).
- C. Differences in FPLC lipoprotein distribution by T1D status in female participants (Female T1D – Female control).
- D. Differences in FPLC lipoprotein distribution by T1D status in male participants (Male T1D – Male control).

Figure 3. Effect of Glucose Control and Insulin Resistance in Altering Lipoprotein Subfraction Patterns in Youth with T1D

A Differences in FPLC lipoprotein distribution youth with T1D by HbA1C. Differences are shown between those subjects at goal (HbA1c<8%) compared to those above goal (HbA1c >8%).

B Differences in FPLC lipoprotein distribution in youth with T1D by Glucose infusion rate (GIR). Differences are shown between subjects above and below the mean GIR.

Table 1

Baseline Characteristic of Participants by T1D Status and Sex

	T1D Male (n=14)	T1D Female (n=14)	Control Male (n=9)	Control Female (n=8)	P-value (Male, T1DvC)	P-value (Female, T1DvC)
Age (years)	15.7 ± 2.2	15.0 ± 1.92	14.6 ± 1.9	15.5 ± 2.3	0.2	0.6
Gender (%)	50%	50%	53%	47%	0.8	0.8
Diabetes Duration (Months)	85.9 ± 59.5	59.3 ± 42.3	NA	NA	NA	NA
Ethnicity (% NHW)	86%	71%	78%	63%	0.6	0.7
Tanner Stage	4.1 ± 0.9	4.5 ± 0.8	3.6 ± 1.1	4.6 ± 0.5	0.2	0.7
Tanner 2	1	0	2	0	NA	NA
Tanner 3	2	2	1	0	NA	NA
Tanner 4	5	3	5	3	NA	NA
Tanner 5	6	9	1	5	NA	NA
BMI (kg/m ²)	20.3 ± 2.3	20.5 ± 3.1	19.6 ± 2.0	20.7 ± 2.4	0.4	0.9
BMI Percentile	43.9 ± 25.3	49.4 ± 25.1	46.8 ± 22.8	52.6 ± 20.2	0.8	0.8
Lean Wt (kg)	47.0 ± 10.8	39.0 ± 4.2	44.9 ± 10.7	35.8 ± 3.7	0.6	0.09
Systolic BP (mm Hg)	118.9 ± 10.2	116.6 ± 13.7	113.4 ± 4.8	108.6 ± 6.9	0.1	0.09
Diastolic BP (mm Hg)	70.0 ± 10.2	69.6 ± 9.3	66.4 ± 7.3	62.4 ± 4.7	0.4	0.03
HbA1C (%)	8.3 ± 1.5	8.4 ± 1.3	5.0 ± 0.4	5.2 ± 0.2	<0.0000	<0.0001
Cholesterol (mg/dL)	149.4 ± 29.5	142.4 ± 40.3	136.1 ± 25.3	148.4 ± 25.0	0.3	0.7
Triglycerides (Geo Mean)	77.9 ± 1.6	70.6 ± 1.5	80.6 ± 1.6	81.5 ± 1.3	0.9	0.4
HDL-C (md/dL)	44.9 ± 8.2	44.1 ± 6.9	46.3 ± 10.0	44.6 ± 7.6	0.7	0.9
LDL-C (mg/dL)	86.9 ± 26.9	82.8 ± 33.0	71.9 ± 25.1	87.0 ± 25.1	0.2	0.7

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

	T1D Male (n=14)	T1D Female (n=14)	Control Male (n=9)	Control Female (n=8)	P-value (Male, T1DvC)	P-value (Female, T1DvC)
Glucose infusion Rate (mg/kg/min)	9.8 ± 2.8	8.5 ± 4.3	16.7 ± 4.4	12.7 ± 2.2	0.0002	0.02