Lipoprotein Subfraction Cholesterol Distribution is Pro-Atherogenic in Women with Type 1 Diabetes and Insulin Resistance

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**Objective:** People with type 1 diabetes have a less atherogenic fasting lipid profile than people without diabetes, but paradoxically have increased rates of cardiovascular disease (CVD). We investigated differences in lipoprotein subfraction cholesterol distribution and insulin resistance (IR) between subjects with and without type 1 diabetes to better understand the etiology of increased CVD risk.

**Research Design and Methods:** Fast protein liquid chromatography was used to fractionate lipoprotein cholesterol distribution in a sub-study of the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study (n=82, age 46±8 years, 52% female, 49% with type 1 diabetes for 23±8 years). IR was assessed by a hyperinsulinemic-euglycemic clamp.

**Results:** Among men, those with type 1 diabetes had less VLDL and more HDL cholesterol than controls (p<0.05), but among women those with diabetes had a shift in cholesterol to denser LDL, *despite more statin use.* Among controls, men had more cholesterol distributed as VLDL and LDL, but less as HDL than women; however, among those with type 1 diabetes, there was no sex difference. Within sex and diabetes strata, a more atherogenic cholesterol distribution by IR was seen in men with and without diabetes, but only in women with type 1 diabetes.

**Conclusion:** The expected sex-based less atherogenic lipoprotein cholesterol distribution was not seen in women with type 1 diabetes. Moreover, IR was associated with a more atherogenic lipoprotein cholesterol distribution in all men, and in women with type 1 diabetes. This lipoprotein cholesterol distribution may contribute to sex-based differences in CVD in type 1 diabetes.
Cardiovascular disease (CVD) is the major cause of mortality in type 1 diabetes and in addition to glycemic control and blood pressure, dyslipidemia is an important and modifiable CVD risk factor(1-8). Curiously, despite higher rates of CVD in type 1 diabetes—including a relative loss of sex-protection in women with type 1 diabetes(9-11)—and the role of dyslipidemia as a determinant of CVD(8), people with type 1 diabetes have similar or less atherogenic lipid profiles than age, sex, and BMI-matched non-diabetics(5;12). This paradox is well-known(13), but few data exist to explain this phenomenon. Lipoprotein differences in type 1 diabetes have been investigated in the DCCT/EDIC study, but lack a non-DM comparison group(14-17).

Furthermore, it has been known for nearly 40 years that insulin resistance (IR) is a prominent CVD risk factor in type 1 diabetes(18;19) and recent studies have demonstrated increased IR in people with type 1 diabetes as compared to age, sex, and BMI matched non-diabetic controls(20;21). The hyperinsulinemic-euglycemic clamp is considered the ‘gold standard’ method for measuring IR in a wide variety of circumstances(22), especially in people with type 1 diabetes, in whom prediction models of IR which rely on glucose and insulin levels cannot be used. The effect of IR on lipoproteins has been investigated in people with type 2 diabetes and without diabetes(23-25), but to our knowledge the effect of IR on lipoproteins has not been investigated in people with type 1 diabetes.

Therefore, to examine beyond the standard fasting lipid profile’s ability to assess CVD risk, we investigated differences in lipoprotein subfraction cholesterol distribution between subjects with and without type 1 diabetes and how IR affects this distribution. We hypothesized that differences would exist in lipoprotein subfraction cholesterol distribution by type 1 diabetes status and furthermore we hypothesized that subjects with more IR as measured by a hyperinsulinemic-euglycemic clamp would have a more atherogenic lipoprotein subfraction cholesterol distribution.

**RESEARCH AND DESIGN METHODS:**

**Study population:** Subjects were recruited from the Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study cohort for a hyperinsulinemic-euglycemic clamp substudy. Inclusion criteria for initial enrollment of type 1 diabetes subjects in the CACTI study were age 19-56, no history of coronary artery disease, on insulin therapy within a year of diagnosis and current insulin therapy, diagnosed before age 30, and diabetes duration ≥ 10 years(4). Non-diabetic (non-DM) controls were recruited from friends and spouses and were of similar age and had no history of coronary artery disease. We present data from 82 subjects (40 type 1 diabetes and 42 non-DM controls) recruited between 2005 and 2008 who underwent a hyperinsulinemic-euglycemic clamp after their six-year CACTI follow up visit. Inclusion criteria for the clamp substudy included HbA1c ≤ 9.5, albumin excretion rate <200 µg/min, and triglycerides <400 mg/dL. Reported physical activity and CT measures of visceral fat from the third CACTI visit (2005-2008) are presented(26). Subjects in the clamp study were similar in age, BMI, visceral fat, and physical activity to subjects in the full cohort seen at the third CACTI visit within sex and diabetes strata. All participants provided informed consent and the study was approved by the Colorado Combined Institutional Review Board.

**Hyperinsulinemic-euglycemic clamp visit:** Subjects were maintained for three days prior to their study day on a provided diet with standardized macronutrient composition (50% carbohydrate, 20% protein, 30% fat) and were asked to refrain from vigorous physical
activity. DEXA scans for body composition measures were performed. Subjects were admitted to the inpatient clinical research unit before a standard dinner the evening before their clamp. Women who were pre-menopausal were scheduled to have their clamp performed between days 2-10 of the menstrual cycle. Type 1 diabetes subjects were instructed to take their last long acting insulin injections at least 12 hours prior to admission. A standard dinner was provided on the unit and subjects were then fasted overnight and through the clamp protocol. Type 1 diabetes subjects received their rapid acting insulin for dinner per their usual regimen. All type 1 diabetes subjects were maintained overnight on intravenous regular insulin with adjustments by a standard protocol to maintain near euglycemia until starting the clamp in the morning. Blood samples for determination of baseline hormone and substrate concentrations (insulin, glucose, C-peptide, FFA, glycerol, and lactate) were drawn over the final 30 minutes before initiation of the clamp protocol. A three stage hyperinsulinemic-euglycemic clamp was then initiated and continued for the next 4.5 hours using the method of DeFronzo et al.(22). Briefly, a primed continuous infusion of insulin was administered at 4 mU/m²/min for 1.5 hours, 8 mU/m²/min for 1.5 hours, and then 40/mU/m²/min for the final 1.5 hours. A variable amount of 20% dextrose was infused to maintain blood glucose ~90 mg/dl. Arterialized blood was sampled every 5 minutes for bedside determination of glucose concentration (Analox, Lunenburg, MA) and the dextrose infusion adjusted as necessary. Arterialized blood samples were taken twice during the last 10 minutes of each stage of the clamp for hormone and substrate measurements as above. A hyperinsulinemic-euglycemic steady-state was achieved in the final 30 minutes of the clamp and glucose infusion rate (GIR) (mg/kg/min) was measured and is presented in these data as mg/kg of fat free mass (FFM)/min. Fasting plasma samples obtained prior to initiation of the clamp were stored at -80°C and then thawed immediately prior to fast protein liquid chromatography (FPLC) analysis in all subjects in the clamp study who had available samples.

**Lipoprotein Analysis:** Individual subject’s EDTA plasma samples (250µL) were chromatographed using two Superose 6 columns in series(27). Fifty-one 0.5mL fractions were collected. Cholesterol was measured in each fraction using a commercially available kit (Cayman Chemical Company) following procedures outlined in the package insert. An example of an individual FPLC lipoprotein subfraction cholesterol distribution profile with fractions labeled as VLDL, LDL, and HDL (increasing density from left to right) is shown in Figure 1.

**Statistical Analyses:** Variables were examined for normality, and non-normally distributed variables were log transformed for analysis. Differences in clinical and clamp parameters between type 1 diabetes and non-DM subjects and between men and women 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 9.2 was used for analyses and Sigma Plot was used for generating difference figures.

**Methods for presentation of Figures:** The cholesterol content in each fraction obtained from the FPLC is expressed as percent of the total cholesterol in all sub-fractions to adjust for differences in total cholesterol levels between subjects adapting the methodology previously used in DCCT/EDIC(14). This is 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 subjects, a difference plot is 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 study subjects are presented in Table 1 stratified by sex and type 1 diabetes status. As previously reported in the full CACTI cohort, type 1 diabetes subjects had a less atherogenic fasting lipid profile than non-diabetics(5). For example, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp. Furthermore, type 1 diabetes subjects were more insulin resistant than non-DM subjects, in both male and female subjects with type 1 diabetes, LDL cholesterol was ~30 mg/dl lower than in non-DM subjects. As expected, type 1 diabetes subjects had a higher pre-clamp fasting glucose and insulin level than non-DM subjects, but there were no differences at the end of the clamp.

Differences by Type 1 Diabetes. The mean lipoprotein subfraction cholesterol distributions are displayed within subjects with type 1 diabetes (Figure 2a, hatched line) and within non-diabetic controls (Figure 2a, solid line). As shown in Figure 2b, subjects with type 1 diabetes had less VLDL and more HDL cholesterol than controls (p<0.05 for both). Next, differences by sex in lipoprotein subfraction cholesterol distribution are displayed within subjects with type 1 diabetes (Figure 3a) and within non-diabetic controls (Figure 3b). Non-DM control men had more cholesterol distributed as VLDL and LDL, but less as HDL than women (p<0.05), as expected, but no significant differences existed by sex in type 1 diabetes subjects.

Differences by Sex. We next investigated differences in lipoprotein subfraction cholesterol distribution by type 1 diabetes status in men (Fig 4a) and women (Fig 4b). Among men, those with type 1 diabetes had less VLDL and more HDL cholesterol than controls. In women, however, those with type 1 diabetes had more LDL cholesterol with an apparent shift of cholesterol distribution within LDL to smaller (atherogenic) LDL fractions in those with diabetes as compared to those without.

Glycemia and Statins. Since statin use and glycemic control are both likely major confounders of lipoprotein subfraction cholesterol distribution differences by diabetes status, we next examined the differences by statin use (yes/no) in type 1 men and type 1 women and then by glycemic control as measured by the highest verses lowest tertiles of A1c (data not shown). No differences in the fasting lipid profile existed within type 1 men and type 1 women based on statin status. LDL cholesterol was similar in men with type 1 diabetes by statin status (72±34 mg/dl v. 69±22 mg/dl, p=0.82) and in women with type 1 diabetes (64±24 mg/dl v. 67±26 mg/dl, p=0.81). However, men with type 1 diabetes in the lowest tertile of A1c (<7.2%) had less cholesterol distributed in LDL as compared to men with type 1 diabetes in the highest A1c tertile (>8.0%), but there
were no differences in VLDL or HDL cholesterol. In women with type 1 diabetes no difference existed in lipoprotein subfraction cholesterol distribution between the lowest A1c tertile (6.9%) and the highest A1c tertile (8.2%).

**Insulin Resistance.** To further investigate these differences and to test the hypothesis that IR affects lipoprotein subfraction cholesterol distribution, we plotted the differences between the highest and lowest tertiles of IR as measured by the hyperinsulinemic-euglycemic clamp protocol within each sex and type 1 diabetes strata. Differences in lipoprotein subfraction cholesterol distribution by these strata were as follows: in males with type 1 diabetes, in the most IR as compared to the least IR there was a shift to increased cholesterol in more dense LDL and HDL (Fig 5a); in male controls, the most IR as compared to the least IR had more VLDL cholesterol as well as a shift to increased cholesterol in denser LDL and HDL (Fig 5b); females with type 1 diabetes in the most IR as compared to the least IR tertile had more cholesterol distributed in VLDL and less distributed in HDL (Fig 5cd); however, in contrast, in female controls, there were no differences in lipoprotein subfraction cholesterol distribution between subjects in the most and least IR tertiles (Fig 5d). Also of note, the pattern for differences in the largest (but not the smallest) lipoprotein HDL cholesterol distribution by IR tertiles for women with type 1 diabetes in Figure 5d was similar to that of males with type 1 diabetes (Fig 5a) and male controls (Fig 5b) by IR tertiles. Therefore, it appears that the effect of IR on lipoprotein subfraction cholesterol distribution was similar in men with and without type 1 diabetes (Figs 5a and 5b) and also in women with type 1 diabetes (Fig 5d), but not in non-DM women (Fig 5c).

**Visceral Fat.** Finally, visceral fat was more strongly correlated with GIR in type 1 subjects (r=-0.51, p=0.017 in females and r=-0.43, p=0.066 in males) than in non-DM subjects (r=-0.29, p=0.19 and r=-0.24, p=0.31 in females and males, respectively.) A modest effect was observed on HDL cholesterol distribution when comparing the highest to lowest visceral fat tertiles within males and females with type 1 diabetes (Figures 6a-d).

**DISCUSSION**

The main findings of this study are that men with type 1 diabetes have a less atherogenic lipoprotein subfraction cholesterol distribution than non-DM men, perhaps due to increased statin use, but women with type 1 diabetes have a more atherogenic distribution as compared to non-DM women with more LDL cholesterol and an apparent shift of cholesterol to a smaller LDL despite the higher prevalence of statin therapy. Furthermore, women with type 1 diabetes have a similar lipoprotein subfraction cholesterol distribution as type 1 men, not the less atherogenic profile that is the expected sex-based difference seen in the non-DM controls. Next, when we investigated the association of IR and lipoprotein subfraction distribution, a pronounced association of higher IR with a more atherogenic lipoprotein subfraction cholesterol distribution was noted among men with and without type 1 diabetes and in women with type 1 diabetes. These data are novel as most previous investigations of lipoprotein distribution in people with type 1 diabetes lacked a non-diabetic control group and furthermore the role of IR has not been investigated. Therefore, these findings suggest that the fasting lipid profile may inadequately assess CVD risk in people with type 1 diabetes and that IR may play an important role in these differences, especially in women with type 1 diabetes.

When we examined the effect of glycemic control in type 1 subjects there was only a minimal effect on lipoprotein subfraction cholesterol distribution (data not shown). The
type 1 subjects had reasonably good glycemic control with a small difference between the highest and lowest A1c tertiles, perhaps explaining this limited association of glycemic control with lipoprotein subfraction cholesterol distribution.

Not unexpectedly, there were no differences in LDL subfraction cholesterol distribution in type 1 subjects based on statin use (data not shown). Previous studies have shown that statins do not change the LDL cholesterol subfraction distribution(28;29) whereas higher doses of a potent statin, i.e. rosuvastatin, can increase buoyant HDL more than small HDL(30). We also did not find a difference in the fasting LDL cholesterol in subjects with type 1 diabetes by statin treatment. It is likely that the type 1 subjects on statin treatment were being treated because of previous lipid abnormalities. Regardless of statin treatment, the type 1 diabetes subjects had mean LDL cholesterol levels near 70 mg/dl. Moreover an effect of statins on LDL cholesterol distribution would be unexpected. In fact this emphasizes the finding of a more atherogenic lipoprotein profile seen in women with type 1 diabetes as compared to non-DM women despite the type 1 women being much more likely to be on statins. In contrast, on the fasting lipid profile, both males and females with type 1 diabetes had LDL-cholesterol ~30 mg/dl lower than non-DM subjects. This highlights how the fasting lipid profile may not adequately represent CVD risk in people with type 1 diabetes. Since only 5 non-DM subjects were on statins, we are unable to compare the effect of statins on lipoprotein subfraction cholesterol distribution between type 1 and non-DM subjects.

Our data on differences in lipoprotein subfraction cholesterol distribution between type 1 and non-DM controls are consistent with previous reports using NMR(31) and of a less atherogenic lipid profile in people with type 1 diabetes as compared to non-DM controls(5;12;13). Furthermore, as measurement of IR in type 1 diabetes requires a clamp, the CACTI clamp substudy provided a unique opportunity to investigate the reported association of lipoprotein cholesterol distribution with IR in persons with type 1 diabetes to extend previous research(23-25) performed in subjects without diabetes and those with type 2 diabetes. For example, our results on the differences by diabetes status, or the differences by IR are greater than the <1% differences reported by Purnell et al in the DCCT/EDIC cohort when the 1st and 4th quartiles of weight gain (a surrogate of IR) were compared within treatment arms(14). The differences we report in lipoprotein subfraction cholesterol distribution related to the differences in IR are similar to or greater than the magnitude of the differences in cholesterol subfractions reported in the DCCT/EDIC cohort between the intensively and conventionally treated arms of the study in which there was a 2% difference in A1c. However, unlike the DCCT, the CACTI study is an observational cohort. Thus, the association of glycemia, obesity, and IR to lipoprotein metabolism and CVD are complex in type 1 diabetes and require further investigation.

Further data from the DCCT/EDIC study using NMR lipoprotein analysis show that male gender and poor glycemia are associated with a more atherogenic lipoprotein profile(16) which is in turn associated with increased carotid IMT(17) and renal dysfunction(32). In contrast, Colhoun has reported a lack of association between lipoprotein subclasses and particle size with coronary artery calcium in adults with type 1 diabetes. The type 1 diabetes subjects had more large and less small HDL than non-DM subjects(31), although we observed this HDL effect only in males. Of note, in this study among people with type 1 diabetes, a sex difference in lipoproteins was also reported; women with type 1 diabetes had less large and more small dense LDL and reduced LDL size.
and this effect of type 1 diabetes on LDL size was significantly different in women than in men(31). Also, our data are consistent with those reported by Colhoun and uniquely extend this finding by implicating IR as part of the pathophysiologic mechanism of sex differences in lipoproteins.

Additional data exist on lipoproteins and IR in people with type 2 diabetes. Goff et al have reported data from the IRAS family study using NMR technology and an IVGTT to assess the associations between IR and lipoproteins in a mixed race cohort with type 2 diabetes, impaired glucose tolerance, and normal glucose tolerance. They reported associations of IR with a variety of lipoprotein measures (size and subclass concentrations) and using factor analysis accounted for 41% of the variance across lipoprotein measures, and which was correlated with IR(24).

Similarly, Garvey et al(23) reported data on the association of glucose disposition rate as determined by a hyperinsulinemic-euglycemic clamp to lipoprotein sizes and concentrations (as measured by NMR) in insulin sensitive, insulin resistant, and untreated type 2 diabetes subjects. IR, though not type 2 diabetes per se, was associated with more atherogenic lipoprotein size and subclass particle concentrations for VLDL, LDL, and HDL, but these differences were not apparent in conventional fasting lipid profiles(23).

Although lipid levels in patients with type 1 diabetes have been found to be comparable to or better than in non-diabetic controls (lower TC, LDL, and TG and higher HDL)(5;12;13), adults with type 1 diabetes still commonly have dyslipidemia and are known to be at higher risk for atherosclerotic disease and for worse outcomes of CVD compared to the general population(7). Dyslipidemia is clearly a major risk factor for atherosclerosis and CVD in adults with both type 1 diabetes and type 2 diabetes(8). The NCEP considers the presence of diabetes to be the risk equivalent of a history of coronary disease with similar goals for lipid lowering(33). While the effectiveness of statin treatment on elevated LDL cholesterol in adults with type 2 diabetes is well-established(8), no clinical trials exist in persons with type 1 diabetes demonstrating LDL cholesterol reduction results in improved CVD outcomes. However, the Heart Protection Study included 615 subjects with type 1 diabetes and the magnitude of reduction in CVD events was similar in type 1 as compared to type 2 diabetes subjects, although underpowered to be statistically significant in subgroup analysis(34).

There is consideration that lipids may be more atherogenic in those with diabetes. Possible mechanisms include differences in lipoprotein particle size, LDL oxidation, and increased transvascular and macrophage LDL transport in patients with type 1 diabetes(31;33;35).

IR is a well-known component of type 2 diabetes, but not always considered as part of type 1 diabetes. Despite the relative lack of dyslipidemia and obesity in type 1 diabetes patients, studies using the hyperinsulinemic-euglycemic clamp have demonstrated increased IR in type 1 diabetes patients when compared to non-DM persons(20;21;36-39) and glucose disposal rate was statistically significantly associated with TG (r=-0.51, p<0.01) but not HDL-c (r=0.13) in a clamp study with 24 subjects with type 1 diabetes(40). It has been proposed that this increased IR may be due to the subcutaneous delivery of insulin in supraphysiologic doses in patients with type 1 diabetes(39). This treatment modality may increase peripheral IR, but would not be expected to affect the liver and lipoprotein production since subcutaneous insulin does not expose the liver to high insulin levels as does endogenous insulin production in hyperinsulinemic type 2 diabetes patients and non-diabetic persons. In the general population, IR is an important component of accelerated atherosclerosis(41).

It is plausible that CAD develops earlier in type 1 diabetes persons who are insulin
resistant(26;42;43). However, direct measurement of IR in insulin-treated patients is difficult and requires the euglycemic insulin clamp approach with careful stabilization of glycemia. Glucose clamps have good intra-subject reproducibility, but are time-consuming, costly and labor intensive, so therefore difficult to perform in large epidemiologic trials. Methods for estimating IR in non-diabetic persons (HOMA, Quicki) have been developed, but cannot be applied to patients with type 1 diabetes since they are severely insulin deficient and therefore unable to secrete insulin in response to glycemic challenge. Moreover, fasting plasma insulin levels only reflect their exogenous insulin treatment regimen.

This study does have limitations. Due to the time intensive nature of the hyperinsulinemic-euglycemic clamp protocol, we have complete data on only these 82 subjects and while they are representative of the full CACTI cohort they may not be of other type 1 diabetes populations. However, this study is much larger than most previous clamp studies performed in people with type 1 diabetes and includes a non-DM control group. Also, it is possible that the control women in the least IR tertile were still sufficiently insulin sensitive to not demonstrate a relationship between IR and an effect on lipoprotein cholesterol distribution. However, sex-differences in non-diabetics are well-described with 41%(44) and 45%(45) greater insulin sensitivity reported in women as compared to men, similar to the difference in our non-diabetic subjects. Furthermore, we present data on lipoprotein subfraction cholesterol distribution on fasting samples collected prior to initiation of the hyperinsulinemic clamp only and the effects of insulin infusion on lipoprotein distribution cannot be examined in this study. Additionally, other analytes were not measured in the lipoprotein subfractions such as triglycerides and these may differ by type 1 diabetes and IR and represent future directions to investigate. Finally, the data presented in this study are cross-sectional and so causation of the reported associations cannot be determined.

In conclusion, a less atherogenic lipoprotein profile is seen in men, but not in women with type 1 diabetes. This is in contrast to the well-described sex difference seen in non-DM women who have a less atherogenic lipid and lipoprotein profile and lower rates of CVD, especially prior to menopause. In men with and without type 1 diabetes and in women with type 1 diabetes, IR was associated with a more atherogenic lipoprotein profile. These data suggest that differences in lipoprotein cholesterol distribution may contribute to sex-based differences in CVD risk in type 1 diabetes and that IR may explain some of this increased relative risk in women with type 1 diabetes. In general, our data reflect the important need for further investigation of the contribution of IR to lipoprotein metabolism and CVD risk in patients with type 1 diabetes.

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FIGURE LEGENDS:

**Figure 1** FPLC lipoprotein cholesterol distribution in a 49 year old non-diabetic female with the following fasting lipid panel of: TC=152, LDL-c=84, HDL-c=53, TG=74 and not on statin therapy.

**Figure 2a** Means of FPLC lipoprotein cholesterol distribution in Subjects with Type 1 Diabetes (hatched line) and Non-Diabetic Controls (solid line)

**Figure 2b** Differences in FPLC lipoprotein distribution by Type 1 Diabetes Status (Type 1 – Non Diabetic, so that a mean above the zero indicates more cholesterol in Type 1 subjects and a mean below the zero line indicates less). Note: Arrows indicate fractions in which statistically significant differences exist.

**Figure 3a** Differences in FPLC lipoprotein distribution by Sex in Type 1 Diabetes Subjects (Male Type 1 – Female Type 1)

**Figure 3b** Differences in FPLC lipoprotein distribution by Sex in Non-Diabetic Subjects (Male NonDM – Female NonDM)

**Figure 4a** Differences in FPLC lipoprotein distribution by Type 1 Diabetes in Male Subjects (Male Type 1 – Male NonDM)

**Figure 4b** Differences in FPLC lipoprotein distribution by Type 1 Diabetes in Female Subjects (Female Type 1 – Female NonDM)

**Figure 5a** Differences in FPLC lipoprotein distribution by Insulin Resistance (highest v. lowest tertiles) in Male Type 1 Diabetes Subjects

**Figure 5b** Differences in FPLC lipoprotein distribution by Insulin Resistance (highest v. lowest tertiles) in Male Non-Diabetic Subjects

**Figure 5c** Differences in FPLC lipoprotein distribution by Insulin Resistance (highest v. lowest tertiles) in Female Type 1 Diabetes Subjects

**Figure 5d** Differences in FPLC lipoprotein distribution by Insulin Resistance (highest v. lowest tertiles) in Female Non-Diabetic Subjects

**Figure 6a** Differences in FPLC lipoprotein distribution by Visceral fat (highest v. lowest tertiles) in Male Type 1 Diabetes Subjects

**Figure 6b** Differences in FPLC lipoprotein distribution by Visceral fat (highest v. lowest tertiles) in Male Non-Diabetic Subjects

**Figure 6c** Differences in FPLC lipoprotein distribution by Visceral fat (highest v. lowest tertiles) in Female Type 1 Diabetes Subjects

**Figure 6d** Differences in FPLC lipoprotein distribution by Visceral fat (highest v. lowest tertiles) in Female Non-Diabetic Subjects

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Table 1 Baseline Characteristic of Subjects by Type 1 Diabetes Status and Sex

|                        | Female: T1D N=21 | Control N=22 | p-value | Male: T1D N=19 | Control N=20 | p-value |
|------------------------|------------------|--------------|---------|----------------|--------------|---------|
| Age, years             | 44±9             | 44±8         | 0.75    | 47±10          | 47±6         | 0.89    |
| T1D duration, years    | 22±4             | NA           | NA      | 23±4           | NA           | NA      |
| GIR, mg/kg FFM/min     | 6.2±3.4          | 15.5±4.8     | <0.0001 | 5.4±3.7        | 10.0±5.2     | 0.004   |
| HbA1c, %               | 7.5±0.9          | 5.4±0.3      | <0.0001 | 7.5±0.8        | 5.4±0.3      | <0.0001 |
| Fasting glucose, mg/dl | 109±19           | 92±6         | 0.0008  | 124±53         | 99±9         | 0.06    |
| Final clamp glucose, mg/dl | 89±4  | 89±4        | 0.90    | 89±2           | 91±3         | 0.10    |
| Baseline insulin, µU/ml| 36±35            | 8±2          | 0.001   | 27±16          | 10±5         | 0.0002  |
| Final clamp insulin, µU/ml | 108±40 | 108±33      | 0.96    | 104±31         | 87±23        | 0.06    |
| Body fat, %            | 32.4±6.7         | 34.7±6.6     | 0.27    | 24.3±6.1       | 24.2±3.2     | 0.92    |
| BMI, kg/m²             | 25.8±4.3         | 25.8±4.3     | 0.99    | 28.3±4.3       | 27.2±3.6     | 0.41    |
| Visceral fat, cm²      | 10.5±0.6         | 10.5±0.3     | 0.93    | 10.9±0.4       | 11.0±0.5     | 0.31    |
| Physical activity, logkcal/s | 7.2±1.3 | 7.4±1.1     | 0.52    | 7.3±1.0        | 7.5±0.8      | 0.50    |
| Total cholesterol, mg/dl| 135±33           | 171±33       | 0.0009  | 145±32         | 171±25       | 0.007   |
| HDL-cholesterol, mg/dl | 56±13            | 57±10        | 0.74    | 61±30          | 45±9         | 0.03    |
| Triglycerides, mg/dl   | 69±42            | 99±40        | 0.02    | 70±22          | 126±73       | 0.003   |
| LDL-cholesterol, mg/dl | 66±25            | 95±29        | 0.0009  | 70±25          | 101±25       | 0.0004  |
| On statins, %          | 52%              | 9%           | 0.002   | 68%            | 15%          | 0.001   |
| OCP use, %             | 86%              | 73%          | 0.46    | NA             | NA           | NA      |
| Post-menopausal, %     | 19%              | 32%          | 0.49    | NA             | NA           | NA      |

*Mean ± SD or frequency

Fig 1
Figure 5a

![Graph showing % Cholesterol vs Fraction number for VLDL, LDL, and HDL](image)

Figure 5b

![Graph showing % Cholesterol vs Fraction number for VLDL, LDL, and HDL](image)
