Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: The CACTI study

Irene E. Schauer, MD, PhD, Janet K. Snell-Bergeon, PhD, Bryan C. Bergman, PhD, David M. Maahs, MD, Adam Kretowski, MD, Robert H. Eckel, MD, and Marian Rewers, MD, PhD

Abbreviated title: Insulin resistance in Type 1 Diabetes

1 Division of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Colorado Denver
2 Barbara Davis Center for Childhood Diabetes, School of Medicine, University of Colorado Denver, Aurora CO

Address all correspondence to:
Irene E. Schauer
Email: Irene.schauer@ucdenver.edu

Submitted 5 March 2010 and accepted 14 October 2010.
**Objective:** To assess insulin action on peripheral glucose utilization and non-esterified fatty acid (NEFA) suppression as a predictor of coronary artery calcification (CAC) in patients with type 1 diabetes and non-diabetic controls.

**Research Design and Methods:** Insulin action was measured by a three-stage hyperinsulinemic-euglycemic clamp (4, 8 and 40mU/m²/min) in 87 subjects from the Coronary Artery Calcification in Type 1 Diabetes cohort (40 diabetic, 47 non-diabetic; mean age 45±8 years; 55% female).

**Results:** Peripheral glucose utilization was lower in subjects with type 1 diabetes compared to non-diabetic controls: glucose infusion rate (mg/kgFFM/min) = 6.19 ± 0.72 \( \text{vs.} \) 12.71±0.66, mean±SE, \( p<0.0001 \), after adjustment for age, sex, BMI, fasting glucose, and final clamp glucose and insulin. Insulin-induced NEFA suppression was also lower in type 1 diabetic compared to non-diabetic subjects: NEFA levels (\( \mu M \)) during 8 mU/m²/min insulin infusion = 370±27 \( \text{vs.} \) 185±25, \( p<0.0001 \), after adjustment for age, sex, BMI, fasting glucose and time point insulin. Lower glucose utilization and higher NEFA levels, correlated with CAC volume (\( r=-0.42 \), \( 95\% \text{CI}=0.22-0.93 \), \( p=0.03 \) and \( r=0.41 \), \( 95\% \text{CI}=1.08-5.32 \), \( p=0.032 \), respectively) and predicted the presence of CAC (OR=0.45, 95%CI=0.22-0.93, \( p=0.03 \); OR=2.4, 95%CI=1.08-5.32, \( p=0.032 \), respectively). Insulin resistance did not correlate with GHb or continuous glucose monitoring parameters.

**Conclusions:** Type 1 diabetic patients are insulin resistant compared to non-diabetic subjects, and the degree of resistance is not related to current glycemic control. Insulin resistance predicts the extent of coronary artery calcification and may contribute to the increased risk of cardiovascular disease in patients with type 1 diabetes as well as subjects without diabetes.

Cardiovascular disease (CVD) remains the leading cause of death in individuals with type 1 diabetes(1-4). While hyperglycemia appears to be the primary mediator of microvascular disease(5; 6), its role in macrovascular disease is less clear(4). Tight glycemic control improves, but does not normalize CVD risk, and correlation of GHb to CVD risk remains controversial(7-15). In addition, standard prediction rules for CVD risk do not accurately predict CVD in type 1 diabetic populations(16). Thus, the mechanism of accelerated atherosclerosis in type 1 diabetes is unclear and identification of those patients at highest risk and most in need of aggressive risk factor modification is inaccurate.

In the general population, insulin resistance has been implicated as an important contributor to accelerated atherosclerosis(17-25). While type 1 diabetes is primarily a disease of insulin deficiency, previous studies have demonstrated insulin resistance and suggested that CVD may also be linked to insulin resistance in type 1 diabetes(10; 26-32). As early as 1968, Martin et al demonstrated an “impaired glucose assimilation index” and an inverse association between this index and prevalent macrovascular disease in type 1 diabetic subjects (30). More recently, the Pittsburgh Epidemiology of Diabetes Complications Study, found no correlation between GHb and coronary artery disease outcomes. However, in addition to other known CVD risk factors, estimated glucose disposal rate correlated inversely with these outcomes(10). Similar correlations of estimated insulin resistance or
a surrogate of insulin resistance (waist to hip ratio) to coronary artery disease were also found in DCCT and EURODIAB(33). These data suggest that an estimate of insulin resistance may add to CVD risk prediction in type 1 diabetes. In addition, elevated non-esterified fatty acid (NEFA) levels have been proposed to mediate the increased atherosclerotic risk associated with insulin resistance in the general population (18; 34-37). It is not known whether the defects in insulin action in type 1 diabetes extend beyond glucose utilization to NEFA suppression.

The Coronary Artery Calcification in Type 1 Diabetes (CACTI) study has followed a cohort of type 1 diabetic subjects and similar non-diabetic controls with Electron Beam CT for measurement of coronary artery calcification (CAC) and CVD outcomes for 6 years(15; 38). We hypothesized that type 1 diabetic subjects would be more insulin resistant than non-diabetic controls in terms of both glucose utilization and NEFA suppression and that both measures of insulin resistance would correlate with CAC, a marker of the extent of coronary atherosclerosis.

RESEARCH DESIGN AND METHODS:
Study population and clamp substudy design: Subjects were recruited from the CACTI study cohort described previously(38) for a hyperinsulinemic-euglycemic clamp sub-study. Inclusion criteria for initial enrollment of type 1 diabetic subjects in the CACTI study were age 19-56, no history of CVD, insulin therapy within a year of diagnosis and current insulin therapy, diagnosis before age 30 and/or positive antibodies, and diabetes duration ≥10 years. The non-diabetic controls were of similar age and free of CVD. Within this cohort, inclusion criteria for the clamp sub-study presented here included: GHb ≤9.5%, albumin excretion rate <200µg/min, triglycerides <400mg/dL, blood pressure <160/100 and a CAC measurement at the 6-year CACTI follow up. Recruitment of the sub-group was by mailing to the full CACTI cohort with the 6-year follow up reminder. The initial recruitment goal was 96 subjects, 24 each of type 1 diabetic men and women and non-diabetic men and women with frequency matching for age (by 5 year intervals) and BMI (normal, overweight, and obese). Interested and eligible members of the cohort were accepted into the sub-study as long as groups remained open and frequency matching criteria were satisfied. Recruitment was stopped at 91 for funding reasons. Clamps were performed 10-967 (median 207) days after the 6-year follow-up visit. All participants provided informed consent and the study was approved by the Colorado Combined Institutional Review Board.

Body Composition: Dual X-ray Absorptiometry scans were performed for body composition and fat free mass just prior to the clamp study. Anthropometric measures included waist and hip circumference, height, weight, and sagittal diameter.

Continuous glucose monitoring: Type 1 diabetic subjects (n=40) underwent masked continuous glucose monitoring (CGM, Medtronic MiniMed Gold System) for three days prior to the hyperinsulinemic-euglycemic clamp. Reported CGM measures include (1) The overall mean glucose (meanT), the average of all glucose values, (2) Hypoglycemia, percentage of glucose values <70mg/dl (3.9mmol/L), (3) Hyperglycemia, percentage of glucose values >180mg/dl (10mmol/L), and (4) Glycemic variability within days, overall standard deviation (SDT), the standard deviation of all glucose values.
Hyperinsulinemic-euglycemic clamp visit:
Subjects were asked to refrain from vigorous physical activity and provided a standardized diet (50% carbohydrate, 20% protein, 30% fat) for three days prior to their study day. Caloric needs were based on fat free mass measured by DEXA scan and a standard activity factor. Premenopausal women were scheduled for their study day within days 2-10 of their menstrual cycle. Subjects were admitted to the inpatient clinical research unit before dinner the evening before their study. Type 1 diabetic subjects were instructed to take their last long acting insulin injections at least 12 hours prior to admission. Dinner was provided on the unit and subjects then fasted overnight and through the clamp. Type 1 diabetic subjects were given bolus insulin for dinner per their usual regimen. Those on an insulin pump had the pump removed after dinner, and all type 1 diabetic subjects were maintained overnight on intravenous insulin with adjustments to achieve euglycemia by morning. Blood samples for determination of baseline hormone and substrate (insulin, glucose, C-peptide, NEFA, glycerol, and lactate) concentrations were drawn before initiation of the clamp protocol. NEFA were assayed using an Olympus AU400e Chemistry Analyzer two step spectrophotometric assay. A three stage hyperinsulinemic- euglycemic clamp was initiated and continued for the next 4.5 hours using the method of DeFronzo et al (39). Briefly, a primed continuous infusion of insulin was administered at 4, 8, and then 40 mU/m²/min for 1.5 hours each. A variable infusion 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, Lunenberg, 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 during the last 30 minutes of the high insulin infusion stage and mean glucose infusion rate (GIR, mg/kg fat-free mass (FFM)/min) during this time was used as the measure of insulin sensitivity.

Imaging of Coronary Artery Calcium:
CAC was measured using an Imatron C-150 Ultrafast CT scanner. All participants underwent two electron beam CT (EBCT) scans without contrast within 5 minutes at baseline and two scans at follow-up visits at years 3 and 6 using the standard acquisition protocol. Calcium volume scores (CVS) were calculated using the volumetric method, which is based on isotropic interpolation, as previously described (40; 41).

Statistical Analyses:
Variables were examined for normality, and non-normally distributed variables were log transformed for analysis. Differences in clinical and clamp parameters and unadjusted GIR between type 1 diabetic and non-DM subjects were examined using unpaired Student's t tests. Multiply adjusted least square means of GIR and NEFA and glycerol levels were calculated using generalized linear models. Correlation of insulin resistance measures to CAC was examined using Spearman rank test and partial adjustment was made for age, diabetes status and sex. Comparison of insulin resistance by GHb quartile was done by ANOVA.

RESULTS
Study Population. We present data from 87 subjects (40 with type 1 diabetes and 47 age and gender matched non-diabetic controls) recruited for the clamp sub-study between 2005 and 2008. Initial recruitment included 91 subjects (44 with diabetes and 47 non-diabetic controls). Four diabetic subject clamps were excluded from the final analysis due to errors in clamp and/or overnight insulin preparation detected by inappropriate insulin responses in the subjects and post-
clamp measurement of infusate insulin concentration.

Subjects in the clamp study were representative of the full CACTI cohort seen at the 6-year follow-up with no differences found for parameters known to impact insulin sensitivity including age (years: 44.8±7.9 vs. 45.0±9.1, p=0.87), BMI (kg/m²: 26.2±4.2 vs. 26.8±4.9, p=0.19), visceral fat (log visceral fat area: 10.67±0.52 vs. 10.74±0.59, p=0.32), and habitual daily physical activity (logkcal: 7.28±1.00 vs. 7.15±1.45, p=0.24). Similarity between the clamp cohort and the full 6-year visit cohort was also found within sex and diabetes strata (not shown). In addition, type 1 diabetic subjects in the clamp study were similar to the full CACTI cohort in diabetes duration (28.6±8.0 vs. 29.4±8.8 years, p=0.57).

Clinical characteristics of the study population by diabetes status are shown in Table 1. Type 1 diabetic and non-diabetic groups were well matched for age and BMI. In addition, the two groups were similar for other measures of body composition, including total percent body fat, waist circumference, waist to hip ratio, and habitual physical activity. As expected, type 1 diabetic and non-diabetic groups differed significantly in diabetes-related parameters of GHb and fasting glucose. Total and LDL cholesterol, and triglycerides also differed significantly by diabetes status, with the non-diabetic subjects exhibiting the higher cardiovascular risk phenotype of higher LDL and triglycerides. Statin use was significantly more frequent in type 1 diabetic subjects, but the difference in lipid profile remained after adjustment for statin use (not shown). Type 1 diabetic subjects were more likely to be taking anti-hypertensive medication, but there was no difference in blood pressure between type 1 diabetic and non-diabetic subjects who were not taking medications (not shown). Adiponectin was significantly higher in type 1 diabetic than non-diabetic subjects as has been reported previously (42-45).

Type 1 diabetic subjects in the clamp cohort exhibited stable glycemic control over the full duration of the study from baseline through the clamp visit (mean GHb=7.71±1.0, 7.57±1.1, 7.9±1.0, and 7.51±0.87 at baseline, 3-year, 6-year, and clamp visits, respectively).

**Impaired peripheral glucose utilization.** Three stage hyperinsulinemic-euglycemic clamps were performed in 87 subjects (46% with type 1 diabetes, 55% women). Final clamp glucose and insulin levels were not different between the groups (Table 2). Whole body insulin sensitivity represented by glucose infusion rate (GIR) during the last 30 minutes of the clamp was significantly lower in type 1 diabetic than non-diabetic subjects (5.8±3.5 vs. 13.2±5.7mg /kg fat free mass (FFM)/min, p<0.0001). This difference was only modestly attenuated after multivariate adjustment for age, fasting glucose, final clamp glucose and insulin, and BMI or visceral fat area (Table 2).

**Correlates of insulin resistance in type 1 diabetes.** Insulin resistance in type 1 diabetes correlated with the expected parameters known to predict insulin resistance in non-diabetic and type 2 diabetic subjects, but relationships were left-shifted in type 1 diabetes. For instance, triglyceride levels and BMI correlated with insulin resistance similarly in both groups (Figure 1). In an analysis of the whole group there was no interaction by diabetes for the relationship of GIR to triglycerides or BMI (p=0.966 and 0.734, respectively), but y-intercepts were significantly different for triglycerides and trended towards significance for BMI (p=0.002 and 0.156, respectively). Similar relationships were found for waist circumference, visceral fat area, and total body fat (not shown).

**Impaired insulin-mediated NEFA suppression.** Insulin-mediated serum NEFA suppression was also impaired in type 1
diabetic subjects (Figure 2, top panel). The increase in serum NEFA levels during the first clamp stage in diabetic subjects reflected an initial insulin infusion rate (4mU/m²/min) that was lower than their basal requirement. The second stage insulin infusion rate (8mU/m²/min) was sufficient to lower glucose and NEFA levels in all type 1 diabetic subjects. Despite this, NEFA levels remained significantly higher in type 1 diabetic than non-diabetic subjects at the end of the second clamp stage (least square mean ± SE: 370±27 vs. 185±25 μM, p<0.0001) after adjustment for age, sex, BMI, fasting glucose, and time point insulin. The highest insulin infusion rate (40mU/m²/min), was sufficient to similarly suppress NEFA levels in type 1 and non-diabetic subjects. NEFA levels at all clamp stages were strongly inversely correlated with peripheral glucose uptake (r=-0.56, p<0.0001, r=-0.63, p<0.0001, r=-0.40, p=0.0002 for stage 1, 2, and 3 NEFA levels, respectively).

Insulin-mediated suppression of glycerol followed a similar pattern to NEFA levels (Figure 1, bottom panel). The defect in glycerol suppression in type 1 diabetic subjects remained significant in the unadjusted data during the third clamp stage, though this was attenuated at the final time point by adjustment for age, sex, BMI, fasting insulin, and time point insulin level.

**Impaired peripheral glucose utilization and insulin-mediated NEFA suppression correlate with coronary artery calcification.** A relationship between insulin resistance and CAC is suggested by a plot of the raw data of peripheral glucose utilization (GIR) vs. CAC volume at the 6-year visit (Figure 3). In fact, after adjustment for age, GIR correlated inversely with CAC volume at the 6-year CACTI follow-up visit and CAC increase from baseline to 6-year and from 3-year to 6-year follow-up visit (Table 3). Partial adjustment for sex and diabetes status attenuated all correlations somewhat (Table 3), but there was no significant interaction with diabetes status. In a logistic regression analysis adjusted for age, sex, BMI, and diabetes status, higher stage 2 NEFA levels (insulin resistance), were independently associated with presence of CAC, odds ratio 2.40, 95% CI (1.08-5.32) for one SD difference in NEFA level. Thus for every 186 μM increase in stage 2 NEFA (signifying insulin resistance), the odds of having CAC increased by 140%. These one standard deviation differences in measures of insulin action are comparable to the differences seen between type 1 diabetic and non-diabetic subjects in this study. In other logistic regression models LDL and HDL levels did not have a significant effect on the odds ratio for presence of CAC in the whole cohort, nor did insulin dose in logistic regression analysis of the diabetes group (not shown).

The above analyses were also performed on the diabetic and non-diabetic groups separately. Similar correlation coefficients resulted from these individual analyses. However, statistical significance was lost for the 3-year to 6-year visit change and otherwise attenuated to near statistical significance in the diabetic group only (Table 3). Similarly, logistic regression analysis of the odds ratio for presence of CAC at the 6-
year follow up visit associated with a change in GIR was analyzed separately for diabetic and non-diabetic subjects. Odds ratios for the two groups were very similar, but statistical significance was lost for the diabetic group [eg, for GIR: Odds ratio = 0.38 (95% CI:0.11-1.37) and 0.40 (95% CI:0.18–0.90), respectively].

**Insulin resistance does not correlate with poor glycemic control.** Neither peripheral glucose uptake nor insulin-mediated NEFA suppression correlated with measures of current or recent glycemic control in this study (Table 4). Pearson coefficients revealed no significant correlation between GIR or stage 2 NEFA levels and GHb or CGM measures of mean glucose, percent of values >180 mg/dl, percent of values <70 mg/dl, and overall SD (glycemic variability).

In addition, GIR and stage 2 NEFA did not differ by GHb quartile (ANOVA, p =0.89; Figure 2 and data not shown) over the range of GHb values represented by this cohort (<9.5%). BMI also did not differ by GHb quartiles (26.4, 28.1, 27.4, and 26.0 kg/m², ANOVA, p=0.27), excluding the possibility that glycemia-related changes in insulin sensitivity were countered by glycemia-related changes in BMI.

**DISCUSSION**

Our study demonstrates significant insulin resistance in subjects with type 1 diabetes relative to non-diabetic controls of similar age, BMI, and habitual physical activity levels. Importantly, type 1 diabetic patients were profoundly insulin resistant despite similar overall adiposity, body fat composition and HDL-cholesterol levels and paradoxically lower fasting triglycerides and higher adiponectin levels. These findings, consistent with previous smaller studies(29; 31)(28), suggest a novel component in the etiology of insulin resistance in type 1 diabetes. Our type 1 diabetic cohort exhibits the expected relationship between insulin resistance and BMI, waist circumference, and triglycerides. However, at any given value the type 1 diabetic subjects are likely to be more insulin resistant than non-diabetic subjects, thus effectively left-shifting the relationship between insulin sensitivity and insulin resistance-associated clinical parameters in type 1 diabetes. Beyond hyperglycemia and the chronic exogenous peripheral hyperinsulinemia of type 1 diabetes, plausible explanations for this “left shift” are lacking.

We have found that in these relatively well controlled type 1 diabetic patients, with mean GHb 7.5%±0.9%, the degree of insulin resistance was not explained by recent glycemic control defined by GHb and CGM measures. Since GHb measured at four points during the study remained consistent, it also does not seem likely that periods of loss of control are responsible for the insulin resistance measured in this study. These data do challenge the general belief that poor glycemic control is the entire etiology of insulin resistance in type 1 diabetes. However, our data do not explicitly contradict existing data. In the Pittsburgh Epidemiology of Diabetes study, where GHb was found to be a significant predictor of insulin resistance, GHb < 9.5-10 defined the low risk group, and a GHb difference representing the full range of our diabetic subjects would result in only a 1 mg/kg/min change in GIR(27). Early studies demonstrating improved GIR with glycemic control involved improvement from very poor control. Our entire diabetic clamp cohort was controlled to the level of the improved GHb in these previous reports. Finally, in the important 1986 study of Yki-Jarvinen, the authors found improved insulin sensitivity with initiation of treatment and improved glycemic control in newly diagnosed type 1 diabetes. However, the scatter plots in this report indicate that improved mean GIR was largely driven by subjects who no longer needed insulin at the 3 month and one year follow ups. Including only the insulin-
dependent subjects at these visits appears to yield a remarkably consistent 40% reduction in GIR across GHb values from 8.8 to 13% and durations of diabetes from 2 weeks to 20 years (46, 47). These results are more consistent with some aspect of non-physiological insulin delivery rather than glycemic control as the etiology of insulin resistance. Our data do not, however, rule out the possibility that very poor glycemic control may contribute to insulin resistance.

We also make the novel observation that insulin resistance in type 1 diabetic subjects also included impaired insulin-mediated suppression of serum NEFA and glycerol levels. The parallel lack of suppression of NEFA and glycerol levels suggests that lipolysis was the source of most of the NEFA measured. However, the patterns were not identical, suggesting that regulation of re-esterification may also be altered between groups.

Insulin resistance has been strongly implicated as a cardiovascular risk factor in the general population (17-25). Our demonstration of insulin resistance in type 1 diabetic subjects in terms of both glucose and NEFA metabolism suggests that insulin resistance may also contribute to increased CVD risk in type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Study demonstrated a correlation between estimated insulin resistance and coronary artery disease (10). In support of this finding, we report significant associations between directly measured insulin resistance and both presence and progression of CAC in our full cohort, as well as in our non-diabetic subgroup. This is a novel finding, representing the only existing demonstration of a correlation between a direct measure of insulin sensitivity by modern validated methodology and an accepted measure of CVD. The finding of statistical significance with this small study group is surprising and supports the hypothesis that insulin resistance is an important factor in CAC development. Though we failed to achieve statistical significance to the level of p<0.05 in the diabetic group, the near significance (p=0.06-0.09) for the correlation of GIR and NEFA suppression to CAC volume at 6 years and to CAC progression from 0 to 6 years suggests that the relationship between IR and CAC is also present in type 1 diabetes. Furthermore, the striking similarity in correlation coefficients between the diabetic and non-diabetic populations in the separate analyses suggest that the slope of the relationship between IR and CAC is similar in subjects with and without type 1 diabetes. The failure to achieve statistical significance in the diabetic group is a limitation of this study. Because of the near significance and the similarity in correlation coefficients and odds ratios to the statistically significant relationship found in the non-diabetic group, we believe this is most likely a power issue related to the smaller range in insulin resistance values and the smaller subject number. However, we cannot rule out the possibility that the association between insulin resistance and CAC is weaker in individuals with type 1 diabetes than those without diabetes.

Because of the left-shift in the relationship between GIR and metabolic syndrome criteria described above, type 1 diabetic subjects in our study had a phenotype less consistent with “metabolic syndrome” than non-diabetic controls despite being more insulin resistant. For instance, type 1 diabetic subjects had a healthier lipid profile than non-diabetic controls even after adjustment for statin use. Similarly hypertension treatment was more common among type 1 diabetic subjects, but overall blood pressure was not higher even after adjustment for medication use. Body composition did not differ between type 1 diabetic and non-diabetic subjects. Thus, standard characteristics that predict insulin resistance in the general population
were not present in our insulin resistant type 1 diabetic patients. This is consistent with a previous report that standard prediction models of CVD risk (highly dependent on hypertension and lipids) do not accurately predict CVD risk in the type 1 diabetic population(16). We also find that about half the CVD risk in our type 1 diabetic cohort is not predicted by standard CVD risk prediction models (manuscript in preparation). We have reported differences in lipoprotein cholesterol subfraction distribution in this clamp group that may explain some of this excess risk(48). Unfortunately, this suggests that traditional approaches to control these risk factors may be inadequate to slow premature formation of coronary atherosclerosis in type 1 diabetes.

The main limitations of this study are the single time point measurement of insulin action, the late study time of this measurement, and the lack of power to reach significance in the type 1 diabetic cohort. We assumed that the single point measure of insulin resistance reflected a parameter that has been fairly constant over the time of the CACTI follow-up. Supporting this assumption, CACTI follow-up has demonstrated essentially stable weight, glycemic control, and lifestyle over the 6-year study span, but a prospective design with repeat clamp studies would be optimal. We are developing an insulin sensitivity prediction model based on this study, and will apply this model to baseline and follow-up data to allow a prospective analysis of the correlation of estimated insulin resistance with CAC and to increase the power of the analysis in the diabetic cohort.

CONCLUSIONS

In summary, we found that profound insulin resistance in type 1 diabetes extends beyond glucose control to regulation of fat metabolism, may be associated with increased coronary atherosclerosis, and cannot be easily identified using standard clinical predictors, including poor glycemic control. These findings suggest that insulin resistance, possibly through effects on overall NEFA exposure and lipotoxicity, may play a role in the residual risk of CVD in type 1 diabetes, as well as in the absence of diabetes, and thus may represent an important therapeutic target that is not currently considered in type 1 diabetes.

Author Contributions. IES contributed to data collection and analysis and wrote the manuscript, JKS-B contributed to data collection, performed statistical analyses, participated in discussion, and reviewed and edited the manuscript, BCB contributed to study design and data collection and reviewed and edited the manuscript, DM contributed to data collection, participated in discussion, and reviewed and edited the manuscript, AK contributed to data collection, RHE and MR contributed to study design, participated in discussion and reviewed and edited the manuscript.

ACKNOWLEDGMENTS

This study was supported in part by National Institutes of Health National Heart, Lung and Blood Institute grants R01 HL61753 and R01 HL079611, and Diabetes Endocrinology Research Center Clinical Investigation Core P30 DK57516. Dr. Schauer was supported by the NIH Office of Research in Women’s Health, Building Interdisciplinary Research Careers in Women’s Health, BIRCWH K12 Program. Dr. Snell-Bergeon was supported by American Diabetes Association Takeda post-doctoral fellowship 7-09-CVD-06. The study was performed at the Adult General Clinical Research Center (now the Clinical Translational Research Center) at the University of Colorado Denver Anschutz Medical Center supported by the NIH M01 RR000051, at the Barbara Davis Center for Childhood Diabetes in Denver, CO, and at
Colorado Heart Imaging Center in Denver, CO.

Portions of this data have previously been reported in abstract/poster form at the 68th and 69th Scientific Sessions of the American Diabetes Association and the 2008 annual NIH - Building Interdisciplinary Careers in Women’s Health meeting.

No potential conflicts of interest relevant to this article were reported.

The authors would also like to acknowledge the assistance of Dr. Jane E.-B. Reusch, MD, in the Division of Endocrinology, Diabetes, and Metabolism at the University of Colorado Denver in manuscript preparation.

REFERENCES

1. Dorman JS, Tajima N, LaPorte RE, Becker DJ, Cruickshanks KJ, Wagener DK, Orchard TJ, Drash AL: The Pittsburgh Insulin-Dependent Diabetes Mellitus (IDDM) Morbidity and Mortality Study: case-control analyses of risk factors for mortality. Diabetes Care 8 Suppl 1:54-60, 1985

2. Gensini GF, Comeglio M, Colella A: Classical risk factors and emerging elements in the risk profile for coronary artery disease. Eur Heart J 19 Suppl A:A53-61, 1998

3. Krolewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR: Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. Am J Cardiol 59:750-755, 1987

4. Orchard TJ, Costacou T, Kretowski A, Nesto RW: Type 1 diabetes and coronary artery disease. Diabetes Care 29:2528-2538, 2006

5. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 329:977-986, 1993

6. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. N Engl J Med 342:381-389, 2000

7. Forrest KY, Becker DJ, Kuller LH, Wolfson SK, Orchard TJ: Are predictors of coronary heart disease and lower-extremity arterial disease in type 1 diabetes the same? A prospective study. Atherosclerosis 148:159-169, 2000

8. Koivisto VA, Stevens LK, Mattock M, Ebeling P, Muggeo M, Stephenson J, Idzior-Walus B: Cardiovascular disease and its risk factors in IDDM in Europe. EURODIAB IDDM Complications Study Group. Diabetes Care 19:689-697, 1996

9. Lehto S, Ronnemaa T, Pyorala K, Laakso M: Poor glycemic control predicts coronary heart disease events in patients with type 1 diabetes without nephropathy. Arterioscler Thromb Vasc Biol 19:1014-1019, 1999

10. Orchard TJ, Olson JC, Erbey JR, Williams K, Forrest KY, Smithline Kinder L, Ellis D, Becker DJ: Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Care 26:1374-1379, 2003

11. Weis U, Turner B, Gibney J, Watts GF, Burke V, Shaw KM, Cummings MH: Long-term predictors of coronary artery disease and mortality in type 1 diabetes. Qjm 94:623-630, 2001
12. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B: Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 353:2643-2653, 2005

13. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, Genuth S: Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med* 348:2294-2303, 2003

14. Nathan DM, Zinman B, Cleary PA, Backlund JY, Genuth S, Miller R, Orchard TJ: Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005). *Arch Intern Med* 169:1307-1316, 2009

15. Snell-Bergeon JK, Hokanson JE, Jensen L, MacKenzie T, Kinney G, Dabelea D, Eckel RH, Ehrlich J, Garg S, Rewers M: Progression of coronary artery calcification in type 1 diabetes: the importance of glycemic control. *Diabetes Care* 26:2923-2928, 2003

16. Zgibor JC, Piatt GA, Ruppert K, Orchard TJ, Roberts MS: Deficiencies of cardiovascular risk prediction models for type 1 diabetes. *Diabetes Care* 29:1860-1865, 2006

17. Fontbonne A, Eschwege E: Insulin-resistance, hypertriglyceridaemia and cardiovascular risk: the Paris Prospective Study. *Diabet Med* 17:93-95, 1991

18. Ginsberg HN: Insulin resistance and cardiovascular disease. *J Clin Invest* 106:453-458, 2000

19. Haffner SM: Epidemiology of insulin resistance and its relation to coronary artery disease. *Am J Cardiol* 84:11J-14J, 1999

20. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 24:683-689, 2001

21. Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R: Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 93:1809-1817, 1996

22. Saely CH, Aczel S, Marte T, Langer P, Hoefle G, Drexel H: The metabolic syndrome, insulin resistance, and cardiovascular risk in diabetic and nondiabetic patients. *J Clin Endocrinol Metab* 90:5698-5703, 2005

23. Hanley AJ, Williams K, Stern MP, Haffner SM: Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. *Diabetes Care* 25:1177-1184, 2002

24. Jeppesen J, Hansen TW, Rasmussen S, Ibsen H, Torp-Pedersen C, Madsbad S: Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease: a population-based study. *J Am Coll Cardiol* 49:2112-2119, 2007

25. Bonora E, Formentini G, Calcaterra F, Lombardi S, Marini F, Zenari L, Saggiani F, Poli M, Perbellini S, Raffaelli A, Cacciatori V, Santi L, Targher G, Bonadonna R, Muggeo M: HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. *Diabetes Care* 25:1135-1141, 2002

26. Kilpatrick ES, Rigby AS, Atkin SL: Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes: "double diabetes" in the Diabetes Control and Complications Trial. *Diabetes Care* 30:707-712, 2007

27. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ: Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 49:626-632, 2000
28. Nadeau KJ, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, Hull A, Zeitler P, Dranin B, Reusch JE: Insulin Resistance in Adolescents with Type 1 Diabetes and Its Relationship to Cardiovascular Function. *J Clin Endocrinol Metab*, 2009
29. DeFronzo RA, Hendler R, Simonson D: Insulin resistance is a prominent feature of insulin-dependent diabetes. *Diabetes* 31:795-801, 1982
30. Martin FI, Stocks AE: Insulin sensitivity and vascular disease in insulin-dependent diabetics. *Br Med J* 2:81-82, 1968
31. Yki-Jarvinen H, Kiviluoto T, Taskinen MR: Insulin resistance is a prominent feature of patients with pancreatogenic diabetes. *Metabolism* 35:718-727, 1986
32. Yki-Jarvinen H, Koivisto VA: Natural course of insulin resistance in type I diabetes. *N Engl J Med* 315:224-230, 1986
33. Pang TT, Narendran P: Addressing insulin resistance in Type 1 diabetes. *Diabet Med* 25:1015-1024, 2008
34. Carlsson M, Wessman Y, Almgren P, Groop L: High levels of nonesterified fatty acids are associated with increased familial risk of cardiovascular disease. *Arterioscler Thromb Vasc Biol* 20:1588-1594, 2000
35. Jouven X, Charles MA, Desnos M, Ducimetiere P: Circulating nonesterified fatty acid level as a predictive risk factor for sudden death in the population. *Circulation* 104:756-761, 2001
36. Pilz S, Scharnagl H, Tiran B, Seelhorst U, Wellnitz B, Boehm BO, Schaefer JR, Marz W: Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease. *J Clin Endocrinol Metab* 91:2542-2547, 2006
37. Steinberg HO, Baron AD: Vascular function, insulin resistance and fatty acids. *Diabetologia* 45:623-634, 2002
38. Dabelea D, Kinney G, Snell-Bergeon JK, Hokanson JE, Eckel RH, Ehrlich J, Garg S, Hamman RF, Rewers M: Effect of type 1 diabetes on the gender difference in coronary artery calcification: a role for insulin resistance? The Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. *Diabetes* 52:2833-2839, 2003
39. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214-223, 1979
40. Mehrotra R, Budoff M, Hokanson JE, Ipp E, Takasu J, Adler S: Progression of coronary artery calcification in diabetics with and without chronic kidney disease. *Kidney Int* 68:1258-1266, 2005
41. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R: Quantification of coronary artery calcification using ultrafast computed tomography. *J Am Coll Cardiol* 15:827-832, 1990
42. Imagawa A, Funahashi T, Nakamura T, Moriwaki M, Tanaka S, Nishizawa H, Sayama K, Uno S, Iwahashi H, Yamagata K, Miyagawa J, Matsuzawa Y: Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes. *Diabetes Care* 25:1665-1666, 2002
43. Frystyk J, Tarnow L, Hansen TK, Parving HH, Flyvbjerg A: Increased serum adiponectin levels in type 1 diabetic patients with microvascular complications. *Diabetologia* 48:1911-1918, 2005
44. Maahs DM, Ogden LG, Snell-Bergeon JK, Kinney GL, Wadwa RP, Hokanson JE, Dabelea D, Kretowski A, Eckel RH, Rewers M: Determinants of serum adiponectin in persons with and without type 1 diabetes. *Am J Epidemiol* 166:731-740, 2007
45. Saraheimo M, Forsblom C, Fagerudd J, Teppo AM, Pettersson-Fernholm K, Frystyk J, Flyvbjerg A, Groop PH: Serum adiponectin is increased in type 1 diabetic patients with nephropathy. *Diabetes Care* 28:1410-1414, 2005
46. Yki-Jarvinen H, Helve E, Koivisto VA: Hyperglycemia decreases glucose uptake in type I diabetes. *Diabetes* 36:892-896, 1987
47. Yki-Jarvinen H, Makimattila S: Insulin resistance due to hyperglycaemia: an adaptation protecting insulin-sensitive tissues. *Diabetologia* 40 Suppl 2:S141-144, 1997
48. Maahs DM, Hokanson JE, Wang H, Kinney GL, Snell-Bergeon JK, East A, Bergman B, Schauer IE, Rewers M, Eckel RH: Lipoprotein Subfraction Cholesterol Distribution is Pro-Atherogenic in Women with Type 1 Diabetes and Insulin Resistance. *Diabetes*

**Figure Legends:**

**Figure 1:** Correlation of insulin sensitivity to Triglycerides and BMI is retained, but left-shifted, in type 1 diabetes. For the relationship of GIR to triglycerides (top panel) the regression equation in type 1 diabetes is GIR = 8.478 – 0.038 (triglycerides), p=0.022; for non-diabetic subjects GIR = 17.14 – 0.037(triglycerides), p=0.011. In combined analysis p=0.966 for an interaction by diabetes and p=0.002 for the difference in y-intercept. For BMI (bottom panel) in type 1 diabetes GIR = 14.732 – 0.33 (BMI), p=0.008; for non-diabetic subjects GIR = 23.87 – 0.41(BMI), p=0.05. In combined analysis p=0.734 for an interaction by diabetes, p=0.156 for the difference in y-intercept.

**Figure 2:** Insulin-mediated NEFA and glycerol suppression are impaired in type 1 diabetic subjects. NEFA and glycerol values are μM. Data are least square mean ± SE (adjusted for age, sex, BMI, fasting glucose, and time point insulin). ■ type 1 diabetic subjects; ▲ non-diabetic controls; * p<0.0001, † p<0.05.

**Figure 3:** Plot of raw data for CAC volume at 6 year follow up visit as a function of GIR (n=87). Open circles = non-diabetic subjects, close circles = type 1 diabetic subjects.

**Figure 4:** Insulin resistance does not correlate with poor glycemic control. Insulin sensitivity is expressed as glucose infusion rate per fat-free mass (GIR, mg/kg FFM/min) and shown by quartile of GHb measured 3 days prior to the clamp study day. GHb range for each quartile is shown and n = 10 for each quartile. Analysis by ANOVA yields p value of 0.89 for differences between quartiles. Pair-wise comparisons are all NS with p values ranging from 0.49 (2nd and 4th quartiles) to 0.96 (1st and 4th quartiles).
### Table 1: Baseline characteristics for clamp study cohort

|                          | Type 1 diabetes (n = 40) | Controls (n = 47) | p-value |
|--------------------------|--------------------------|-------------------|---------|
| Age (years)              | 45.2 ± 9.2               | 45.9 ± 7.2        | 0.7     |
| Male/female              | 19/21                    | 20/27             | -       |
| Duration of diabetes (years) | 22.6 ± 7.8             | -                 | -       |
| GHb (%)                  | 7.5 ± 0.9                | 5.4 ± 0.3         | <0.0001 |
| Fasting glucose (mg/dl)  | 116 ± 39                 | 96 ± 8            | 0.0024  |
| Body fat (%)             | 28.6 ± 7.5               | 29.6 ± 7.1        | 0.53    |
| BMI (kg/m²)              | 27.0 ± 4.4               | 26.0 ± 4.1        | 0.31    |
| Visceral fat area (cm²)  | 72.7 ± 50.5              | 80.5 ± 50.5       | 0.49    |
| Subcutaneous fat area (cm²) | 271 ± 126              | 273 ± 112         | 0.97    |
| Sagittal diameter (mm)   | 214 ± 36                 | 214 ± 31          | 0.96    |
| Waist circumference (cm) | 88.5 ± 12.3              | 86.3 ± 12.4       | 0.42    |
| Waist:hip ratio          | 0.84 ± 0.08              | 0.83 ± 0.09       | 0.44    |
| Liver:spleen density ratio | 1.26 ± 0.08            | 1.24 ± 0.24       | 0.58    |
| Total cholesterol (mg/dl)| 139.9 ± 32.4             | 171.1 ± 28.8      | <0.0001 |
| HDL-cholesterol (mg/dl)  | 58.0 ± 22.4              | 53.4 ± 15.0       | 0.27    |
| Triglycerides (mg/dl)    | 69.7 ± 33.8              | 108.0 ± 57.2      | 0.0002  |
| LDL-cholesterol (mg/dl)  | 67.6 ± 24.8              | 96.1 ± 26.0       | <0.0001 |
| Systolic blood pressure (mmHg) | 114 ± 11              | 113 ± 10          | 0.61    |
| Diastolic blood pressure (mmHg) | 73 ± 7                | 76 ± 8            | 0.09    |
| On hypertension medications (%) | 45                  | 13                | 0.0005  |
| Habitual physical activity (log kcal) | 7.2 ± 1.2          | 7.4 ± 7.1         | 0.42    |
| Adiponectin (geometric mean ± SD) | 10.9 ± 1.7          | 8.2 ± 1.8         | 0.02    |
| Cortisol (μg/dl)         | 11.8 ± 4.3              | 11.5 ± 4.4        | 0.8     |

Values are unadjusted mean ± SD except where otherwise indicated.

### Table 2: Insulin sensitivity by glucose infusion rate (GIR) from hyperinsulinemic- euglycemic clamp

|                          | Type 1 Diabetes | Non-diabetic Controls | p-value |
|--------------------------|-----------------|-----------------------|---------|
| **Stage 3 clamp parameters:** |                 |                       |         |
| Final glucose (mg/dl)    | 89.1 ± 3.2      | 89.9 ± 3.4            | 0.28    |
| Final insulin (μU/ml)    | 106 ± 35        | 99 ± 30               | 0.36    |
| **Glucose infusion rate (mg/kg FFM/min):** |                 |                       |         |
| Unadjusted (mean ± SD)   | 5.8 ± 3.5       | 13.2 ± 5.7            | <0.0001 |
| LS Mean ± SE, adjusted for age, sex, BMI, fasting glucose, final glucose and insulin | 6.19 ± 0.72 | 12.71 ± 0.66 | <0.0001 |
| LS Mean ± SE, adjusted for age, sex, visceral fat, fasting glucose, final glucose and insulin | 6.24 ± 0.68 | 12.75 ± 0.62 | <0.0001 |
Table 3: Correlation of IR to CAC volume; OR of any CAC at 6-year visit associated with insulin resistance by GIR and by failure of NEFA suppression in clamp stage 2.

| Model                                      | Spearman correlation coefficient: Glucose infusion rate vs. CAC volume for total clamp cohort (n=87) | 6-year visit CAC volume | Baseline to 6-year visit change in CAC | 3-year to 6-year visit change in CAC |
|--------------------------------------------|----------------------------------------------------------------------------------------------------|-------------------------|----------------------------------------|--------------------------------------|
| 1: adjusted for age                        | GIR (mg/kg FFM/min)                                                                                   | -0.42 (p<0.0001)       | -0.41 (p<0.0001)                      | -0.24 (p=0.028)                     |
|                                            | Stage 2 NEFA level (μM)                                                                                | 0.41 (p=0.0001)        | 0.40 (p=0.0001)                      | 0.27 (p=0.01)                      |
| 2: adjusted for age, sex, diabetes status  | GIR (mg/kg FFM/min)                                                                                   | -0.31 (p=0.005)        | -0.30 (p=0.006)                      | -0.19 (p=0.08)                     |
|                                            | Stage 2 NEFA level (μM)                                                                                | 0.32 (p=0.003)         | 0.30 (p=0.005)                      | 0.24 (p=0.03)                      |
| 3: adjusted for age: Type 1 diabetic group alone: | GIR (mg/kg FFM/min)                                                                                   | -0.28 (p=0.08)         | -0.28 (p=0.09)                      | NS                                  |
|                                            | Stage 2 NEFA level (μM)                                                                                | 0.31 (p=0.06)          | 0.28 (p=0.08)                       | NS                                  |
| 4: adjusted for age: Non-diabetic group alone: | GIR (mg/kg FFM/min)                                                                                   | -0.39 (p=0.006)        | -0.41 (p=0.004)                      | NS                                  |
|                                            | Stage 2 NEFA level (μM)                                                                                | 0.39 (p=0.007)         | 0.39 (p=0.008)                      | NS                                  |

Logistic regression analysis: Odds ratio for any CAC at 6-year visit with increase in insulin resistance (n=87)

|                          | Mean ± SD                  | OR per 1 SD change (95% confidence interval) (p-value) |
|--------------------------|-----------------------------|--------------------------------------------------------|
| GIR (mg/kgFFM/min)       | 9.8 ± 6.1                   | 0.45 (0.22-0.93) (0.03)                                 |
| Stage 2 NEFA (μM)        | 268 ± 186                   | 2.40 (1.08-5.32) (0.032)                               |

Table 4: Insulin resistance does not correlate with recent glycemic control. Pearson correlation coefficients are shown for correlation of GIR and stage 2 NEFA levels to GHb and CGM measures of glycemic control for all type 1 diabetic subjects.

|                          | Mean ± SD                  | Correlation to GIR | Correlation to stage 2 NEFA |
|--------------------------|-----------------------------|--------------------|------------------------------|
|                          | r-value | p-value      | r-value | p-value      |
| GHb (%)                  | 7.5 ± 0.9 | 0.13 | 0.5       | -0.02 | 0.92       |
| CGM parameters:          |                     |                   |                     |
| Mean glucose (mg/dl)     | 141 ± 26 | 0.2 | 0.26      | -0.16 | 0.37       |
| % > 180 mg/dl            | 26.2 ± 12.9 | 0.25 | 0.14      | -0.15 | 0.40       |
| % < 70 mg/dl             | 16.2 ± 11.9 | -0.02 | 0.91      | -0.04 | 0.84       |
| Overall SD               | 65.9 ± 20.1 | 0.21 | 0.22      | -0.16 | 0.35       |
Figure 1

Glucose Infusion Rate (mg/kg FFM/min)

- Type 1 diabetes
- Non-diabetes

Triglycerides (mg/dl)

- $y = -0.037x + 17.14$
- $y = -0.038x + 8.48$

Body Mass Index (kg/m²)

- $y = -0.41x + 23.87$
- $y = -0.33x + 14.73$
Figure 2

[Graph showing NEFA and Glycerol levels over clamp time (minutes)].

Figure 3

[Graph showing CAC Volume, square root transformed against Glucose Infusion Rate (mg/kgFFM/min)]

- ▲ Type 1 diabetes
- □ Non-diabetes
Figure 4

A bar chart showing the glucose infusion rate (mg/kgFFM/min) across different hemoglobin A1c quartiles and ranges. The chart indicates a P-value of 0.89.