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,[1] Janet K. Snell-Bergeon,[2] Bryan C. Bergman,[1] David M. Maahs,[2] Adam Kretowski,[1] Robert H. Eckel,[1] and Marian Rewers[2]

OBJECTIVE—To assess insulin action on peripheral glucose utilization and nonesterified fatty acid (NEFA) suppression as a predictor of coronary artery calcification (CAC) in patients with type 1 diabetes and nondiabetic controls.

RESEARCH DESIGN AND METHODS—Insulin action was measured by a three-stage hyperinsulinemic-euglycemic clamp (4, 8, and 40 mU/m^2/min) in 87 subjects from the Coronary Artery Calcification in Type 1 Diabetes cohort (40 diabetic, 47 nondiabetic; mean age 45 ± 8 years; 55% female).

RESULTS—Peripheral glucose utilization was lower in subjects with type 1 diabetes compared with nondiabetic controls: glucose infusion rate (mg/kg FFM/min) = 6.19 ± 0.72 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 with nondiabetic subjects: NEFA levels (μM) during 8 mU/m^2/min insulin infusion = 370 ± 27 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 ($r = -0.42$, $P < 0.0001$ and $r = 0.41$, $P < 0.0001$, respectively) and predicted the presence of CAC (odds ratio [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 with nondiabetic 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.
***Diabetes* 60:306–314, 2011**

From the [1]Division of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Colorado Denver, Aurora, Colorado; and the [2]Barbara Davis Center for Childhood Diabetes, School of Medicine, University of Colorado Denver, Aurora, Colorado.

Corresponding author: Irene E. Schauer, irene.schauer@ucdenver.edu.

Received 5 March 2010 and accepted 14 October 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 26 October 2010. DOI: 10.2337/db10-0328.

Cardiovascular disease (CVD) remains the leading cause of death in individuals with type 1 diabetes (1–4). Although 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). Although 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. (30) demonstrated an "impaired glucose assimilation index" and an inverse association between this index and prevalent macrovascular disease in type 1 diabetic subjects. More recently, the Pittsburgh Epidemiology of Diabetes Complications Study (10) 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. Similar correlations of estimated insulin resistance or a surrogate of insulin resistance (waist-to-hip ratio) to coronary artery disease were also found in the Diabetes Control and Complications Trial (DCCT) and the EURODIAB study (33). These data suggest that an estimate of insulin resistance may add to CVD risk prediction in type 1 diabetes. In addition, elevated nonesterified 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 nondiabetic controls with electron beam computed tomography 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 nondiabetic 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 substudy. Inclusion criteria for initial enrollment of type 1 diabetic subjects in the CACTI study were age 19 to 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 nondiabetic controls were of similar age and free of CVD. Within this cohort, inclusion criteria for the clamp substudy presented here included: GHb ≤9.5%, albumin excretion rate <200 μg/min, triglycerides <400 mg/dl, blood pressure <160/100, and a CAC measurement at the 6-year CACTI follow-up. Recruitment of the subgroup was performed by a 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 nondiabetic 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 substudy as long as groups remained open and frequency-matching criteria were satisfied. Recruitment was stopped at 91 participants for funding reasons. Clamps were performed 10 to 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 (FFM) just before the clamp study. Abdominal computed tomography scans for calculation of visceral and subcutaneous fat areas and liver to spleen density ratios were performed at the 6-year CACTI follow-up and within 1 year of the clamp study. Anthropometric measures included waist and hip circumference, height, weight, and sagittal diameter.

Continuous glucose monitoring. Type 1 diabetic subjects (n = 44) underwent masked continuous glucose monitoring (CGM) (Medtronic MiniMed Gold System) for 3 days before the hyperinsulinemic-euglycemic clamp. Reported CGM measures include *1*) the overall mean glucose ($mean_T$), the average of all glucose values; *2*) hypoglycemia, percentage of glucose values <70 mg/dl (3.9 mmol/l); *3*) hyperglycemia, percentage of glucose values >180 mg/dl (10 mmol/l); and *4*) glycemic variability within days, overall SD (SD_T), the SD 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 3 days before their study day. Caloric needs were based on FFM measured by DEXA scan and a standard activity factor. Premenopausal women were scheduled for their study day within days 2 to 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 h before 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 h using the method of DeFronzo et al. (39). Briefly, a primed continuous infusion of insulin was administered at 4, 8, and then 40 mU/m^2/min for 1.5 h each. A variable infusion of 20% dextrose was infused to maintain blood glucose ~90 mg/dl. Arterialized blood was sampled every 5 min 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 min of each stage of the clamp for hormone and substrate measurements as above. A hyperinsulinemic- euglycemic steady state was achieved during the last 30 min of the high insulin infusion stage and mean glucose infusion rate ([GIR], mg/kg 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 computed tomography scanner. All participants underwent two electron beam computed tomography scans without contrast within 5 min 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 nonnormally 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 t tests. Multiply adjusted least squares 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

We present data from 87 subjects (40 with type 1 diabetes and 47 age- and sex-matched nondiabetic controls) recruited for the clamp substudy between 2005 and 2008. Initial recruitment included 91 subjects (44 with diabetes and 47 nondiabetic 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 postclamp 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/m^2: 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 (log Kcal: 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 nondiabetic groups were well matched for age and BMI. In addition, the two groups were similar for other measures of body composition, including total percentage of body fat, waist circumference, waist to hip ratio, and habitual physical activity. As expected, type 1 diabetic and nondiabetic 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 nondiabetic 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 antihypertensive medication, but there was no difference in blood pressure between type 1 diabetic and nondiabetic subjects who were not taking medications (not shown). Adiponectin was significantly higher in type 1 diabetic than nondiabetic 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

TABLE 1
Baseline characteristics for clamp study cohort

	Type 1 diabetes (n = 40)	Controls (n = 47)	P
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/m^2)	27.0 ± 4.4	26.0 ± 4.1	0.31
Visceral fat area (cm^2)	72.7 ± 50.5	80.5 ± 50.5	0.49
Subcutaneous fat area (cm^2)	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
Adiponection (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.

clamp glucose and insulin levels were not different between the groups (Table 2). Whole-body insulin sensitivity represented by GIR during the last 30 min of the clamp was significantly lower in type 1 diabetic than nondiabetic subjects (5.8 ± 3.5 vs. 13.2 ± 5.7 mg/kg 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 nondiabetic 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 (Fig. 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 the y intercepts were significantly different for triglycerides and trended toward 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 (Fig. 2, top panel). The increase in serum NEFA levels during the first clamp stage in diabetic subjects reflected an initial insulin infusion rate (4mU/m^2/min) that was lower than their basal requirement. The second stage insulin infusion rate (8mU/m^2/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 nondiabetic subjects at the end of the second clamp stage (least squares mean ± SE: 370 ± 27 vs. 185 ± 25 μmol/l, $P <$ 0.0001) after adjustment for age, sex, BMI, fasting glucose, and time point insulin. The highest insulin infusion rate (40mU/m^2/min), was sufficient to similarly suppress NEFA levels in type 1 and nondiabetic subjects. NEFA levels at all clamp stages were strongly inversely correlated with peripheral glucose uptake ($r = -0.56$, $P < 0.0001$, $r =$

TABLE 2
Insulin sensitivity by glucose infusion rate from hyperinsulinemic-euglycemic clamp

	Type 1 diabetes	Nondiabetic controls	P
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

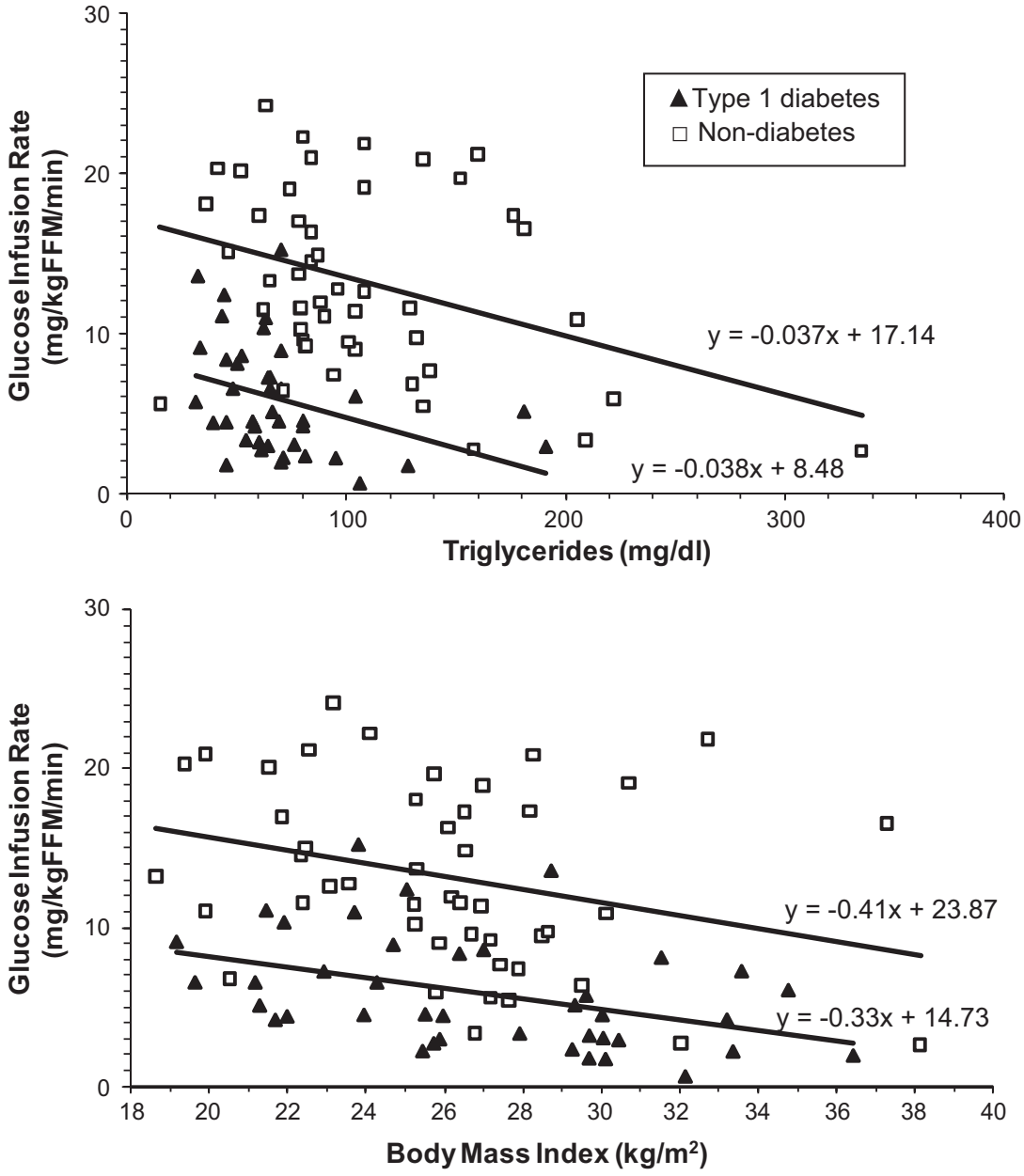

FIG. 1. Correlation of insulin sensitivity to triglycerides and BMI is retained, but left-shifted, in type 1 diabetes. For the relationship of GIR to triglycerides (*top panel*) the regression equation in type 1 diabetes is GIR = 8.478 – 0.038 (triglycerides), $P = 0.022$; for nondiabetic subjects GIR = 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 nondiabetic 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 the y intercept.

-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 (Fig. 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) versus CAC volume at the 6-year visit (Fig. 3). In fact, after adjustment for age, GIR correlated inversely with CAC volume at the 6-year CACTI follow-up visit and with an increase in CAC volume from baseline to the 6-year visit and from 3-year to 6-year follow-up visits (ρ, $P = -0.42$, <0.0001; -0.41, <0.0001; -0.24, <0.028, respectively) (Table 3). In a logistic regression analysis adjusted for age, sex, BMI, and diabetes status, lower GIR was independently associated with the presence of CAC: odds ratio (OR) 0.45, 95% CI (0.22–0.93) for a one standard deviation (SD) increase in GIR. Thus, for every 6.1 mg/kg FFM/min increase in GIR (signifying greater insulin sensitivity), the odds of having CAC decreased by 55%.

The NEFA levels during stage 2 of the clamp were similarly but positively correlated with CAC volume at the 6-year visit, and CAC increase from baseline to 6-year and

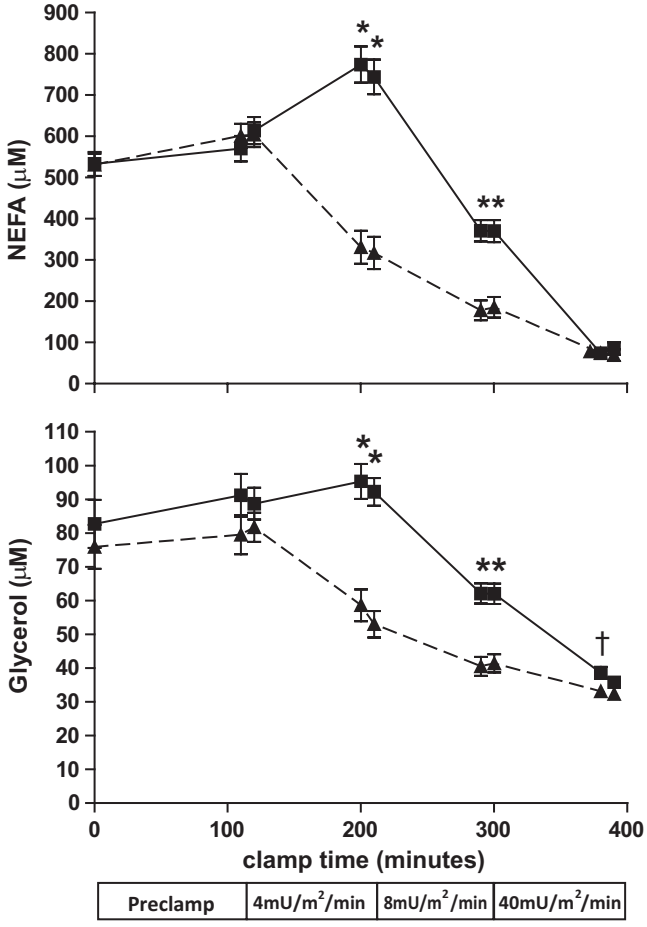

FIG. 2. Insulin-mediated NEFA and glycerol suppression are impaired in type 1 diabetic subjects. NEFA and glycerol values are μM. Data are least squares mean ± SE (adjusted for age, sex, BMI, fasting glucose, and time point insulin). ■, type 1 diabetic subjects; ▲, nondiabetic controls; *$P < 0.0001$,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

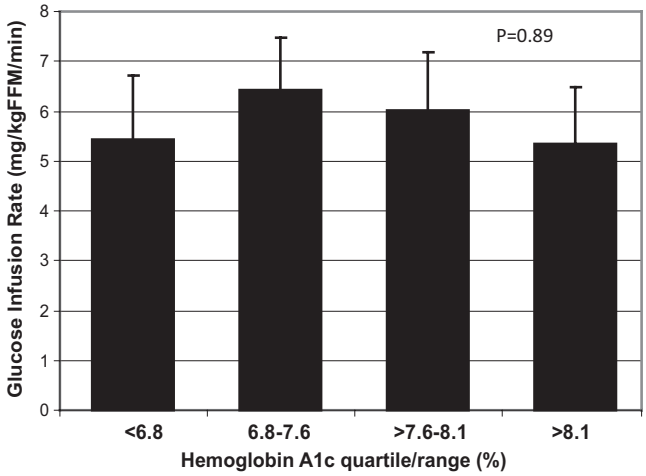

FIG. 4. Insulin resistance does not correlate with poor glycemic control. Insulin sensitivity is expressed as glucose infusion rate per fat-free mass (GIR, mg/kg FFM/min) and shown by quartile of GHb measured 3 days before the clamp study day. GHb range for each quartile is shown and $n = 10$ for each quartile. Analysis by ANOVA yields a P value of 0.89 for differences between quartiles. Pairwise comparisons are all nonsignificant with P values ranging from 0.49 (2nd and 4th quartiles) to 0.96 (1st and 4th quartiles).

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 the presence of CAC, OR 2.40, 95% CI (1.08–5.32) for one SD difference in NEFA level. Thus, for every 186 μmol/l increase in stage 2 NEFA (signifying insulin resistance), the odds of having CAC increased by 140%. These one SD differences in measures of insulin action are comparable to the differences seen between type 1 diabetic and nondiabetic subjects in this study. In other logistic regression models LDL and HDL levels did not have a significant effect on the odds ratio for

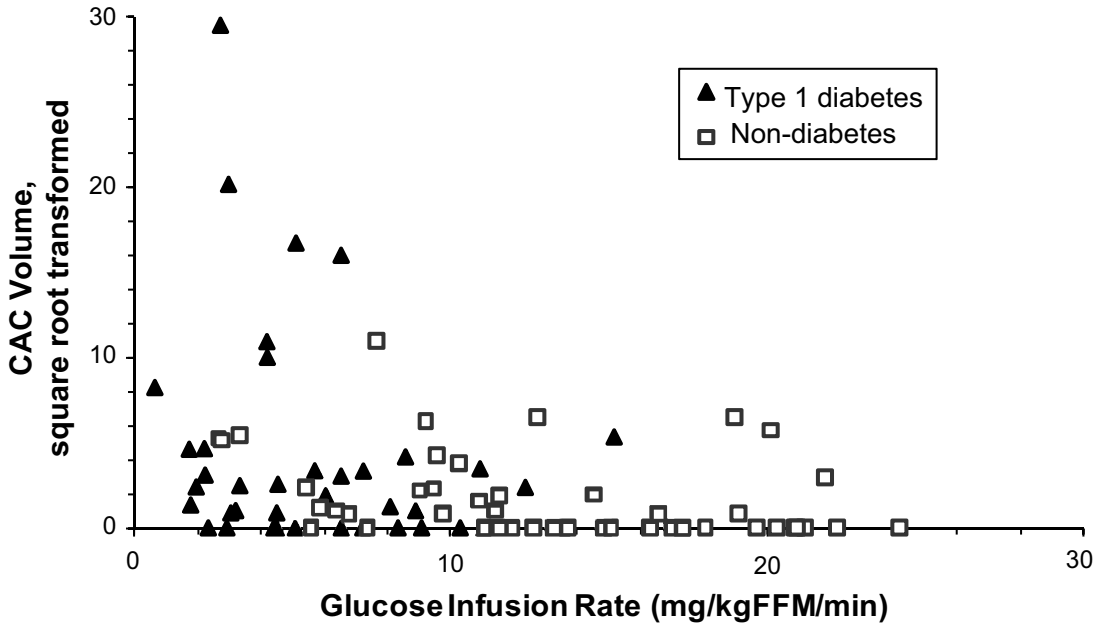

FIG. 3. Plot of raw data for CAC volume at 6-year follow-up visit as a function of GIR ($n = 87$). Open (white) squares = nondiabetic subjects, black triangles = type 1 diabetic subjects.

TABLE 3
Correlation of insulin resistance 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: nondiabetic 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% CI) (P)
GIR (mg/kg FFM/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)

the presence of CAC in the whole cohort, nor did insulin dose in logistic regression analysis of the diabetic group (not shown).

The above analyses were also performed on the diabetic and nondiabetic 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, models 3 and 4). Similarly, logistic regression analysis of the odds ratio for the presence of CAC at the 6-year follow-up visit associated with a change in GIR was analyzed separately for diabetic and nondiabetic subjects. Odds ratios for the two groups were very similar, but statistical significance was lost for the diabetic group (e.g., for GIR: OR = 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, percentage of values >180 mg/dl, percentage of values <70 mg/dl, and overall SD (glycemic variability).

In addition, GIR and stage 2 NEFA did not differ by GHb quartile (ANOVA, $P = 0.89$; Fig. 4 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/m^2, 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 nondiabetic 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 (28,29,31), 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 nondiabetic 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

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

		Correlation to GIR		Correlation to stage 2 NEFA	
	Mean ± SD	r	P	r	P
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

degree of insulin resistance was not explained by recent glycemic control defined by GHb and CGM measures. Since GHb was measured at four points during the study and 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, (46,47) 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 1-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. These results are more consistent with some aspect of nonphysiologic 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 type1 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 nondiabetic 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 insulin resistance and CAC is also present in type 1 diabetes. Furthermore, the striking similarity in correlation coefficients between the diabetic and nondiabetic populations in the separate analyses suggests that the slope of the relationship between insulin resistance 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 nondiabetic 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 nondiabetic controls, despite being more insulin resistant. For instance, type 1 diabetic subjects had a healthier lipid profile than nondiabetic 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 nondiabetic 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 (J.K.S.-B., I.E.S., B.C.B., D.M.M., R.H.E., M.R., unpublished observations). 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.

ACKNOWLEDGMENTS

This study was supported in part by National Institutes of Health National Heart, Lung, and Blood Institute grants R01 HL-61753 and R01 HL-079611, and Diabetes Endocrinology Research Center Clinical Investigation Core P30 DK-57516. I.E.S. was supported by the National Institutes of Health Office of Research in Women's Health, Building Interdisciplinary Research Careers in Women's Health, BIRCWH K12 Program. J.K.S.-B. was supported by an American Diabetes Association Takeda postdoctoral fellowship 7-09-CVD-06.

This 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 National Institutes of Health M01 RR-000051, at the Barbara Davis Center for Childhood Diabetes in Denver, Colorado, and at the Colorado Heart Imaging Center in Denver, Colorado.

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

I.E.S. contributed to data collection and analysis and wrote the manuscript. J.K.S.-B. contributed to data collection, performed statistical analyses, participated in discussion, and reviewed and edited the manuscript. B.C.B. contributed to study design and data collection and reviewed and edited the manuscript. D.M. contributed to data collection, participated in discussion, and reviewed and edited the manuscript. A.K. contributed to data collection. R.H.E. and M.R. contributed to study design, participated in discussion, and reviewed and edited the manuscript.

Portions of these data have previously been reported in abstract/poster form at the 68th and 69th Scientific Sessions of the American Diabetes Association (June 7, 2008 and June 6, 2009) and the 2008 annual National Institutes of Health—Building Interdisciplinary Careers in Women's Health meeting (November 16–17, 2009).

The authors would like to thank Jane E.-B. Reusch, MD, in the Division of Endocrinology, Diabetes, and Metabolism at the University of Colorado Denver for assistance with 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 1985;8(Suppl 1):54–60.
2. Gensini GF, Comeglio M, Colella A. Classical risk factors and emerging elements in the risk profile for coronary artery disease. Eur Heart J 1998;(Suppl A):A53–A61
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 1987;59:750–755
4. Orchard TJ, Costacou T, Kretowski A, Nesto RW. Type 1 diabetes and coronary artery disease. Diabetes Care 2006;29:2528–2538
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 1993;329:977–986
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 2000;342:381–389
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 2000;148:159–169
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 1996;19:689–697
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 1999;19:1014–1019
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 2003;26:1374–1379
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 2001;94:623–630
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 2005;353:2643–2653
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 2003;348:2294–2303
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 2009;169:1307–1316
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 2003;26:2923–2928
16. Zgibor JC, Piatt GA, Ruppert K, Orchard TJ, Roberts MS. Deficiencies of cardiovascular risk prediction models for type 1 diabetes. Diabetes Care 2006;29:1860–1865
17. Fontbonne A, Eschwege E. Insulin-resistance, hypertriglyceridaemia and cardiovascular risk: the Paris Prospective Study. Diabete Metab 1991;17:93–95
18. Ginsberg HN. Insulin resistance and cardiovascular disease. J Clin Invest 2000;106:453–458
19. Haffner SM: Epidemiology of insulin resistance and its relation to coronary artery disease. Am J Cardiol 1999;84:11J–14J
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 2001;24:683–689
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 1996;93:1809–1817
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 2005;90:5698–5703
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 2002;25:1177–1184
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 2007;49:2112–2119
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 2002;25:1135–1141
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 2007;30:707–712
27. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical

factors estimate insulin resistance in type 1 diabetes? Diabetes 2000;49:626–632
28. Nadeau KJ, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, Hull A, Zeitler P, Draznin B, Reusch JE. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. J Clin Endocrinol Metab 2010;95:513–521
29. DeFronzo RA, Hendler R, Simonson D. Insulin resistance is a prominent feature of insulin-dependent diabetes. Diabetes 1982;31:795–801
30. Martin FI, Stocks AE. Insulin sensitivity and vascular disease in insulin-dependent diabetics. Br Med J 1968;2:81–82
31. Yki-Jarvinen H, Kiviluoto T, Taskinen MR. Insulin resistance is a prominent feature of patients with pancreatogenic diabetes. Metabolism 1986;35:718–727
32. Yki-Jarvinen H, Koivisto VA. Natural course of insulin resistance in type I diabetes. N Engl J Med 1986;315:224–230
33. Pang TT, Narendran P. Addressing insulin resistance in Type 1 diabetes. Diabet Med 2008;25:1015–1024
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 2000;20:1588–1594
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 2001;104:756–761
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 2006;91:2542–2547
37. Steinberg HO, Baron AD. Vascular function, insulin resistance and fatty acids. Diabetologia 2002;45:623–634
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 2003;52:2833–2839
39. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–223
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 2005;68:1258–1266
41. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–832
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 2002;25:1665–1666
43. Frystyk J, Tarnow L, Hansen TK, Parving HH, Flyvbjerg A. Increased serum adiponectin levels in type 1 diabetic patients with microvascular complications. Diabetologia 2005;48:1911–1918
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 2007;166:731–740
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 2005;28:1410–1414
46. Yki-Jarvinen H, Helve E, Koivisto VA. Hyperglycemia decreases glucose uptake in type I diabetes. Diabetes 1987;36:892–896
47. Yki-Jarvinen H, Makimattila S. Insulin resistance due to hyperglycaemia: an adaptation protecting insulin-sensitive tissues. Diabetologia 1997;40;(Suppl 2):S141–S144
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 proatherogenic in women with type 1 diabetes and insulin resistance. Diabetes 2010;59:1771–1179