

Insulin Resistance in Adolescents with Type 1 Diabetes and Its Relationship to Cardiovascular Function

Kristen J. Nadeau, Judith G. Regensteiner, Timothy A. Bauer, Mark S. Brown, Jennifer L. Dorosz, Amber Hull, Phil Zeitler, Boris Draznin,* and Jane E. B. Reusch*

University of Colorado Denver School of Medicine, Division of Pediatric Endocrinology (K.J.N., A.H., P.Z.), The Children's Hospital; Division of General Internal Medicine (J.G.R.), Center for Women's Health Research; Division of General Internal Medicine (T.A.B.); Division of Radiology (M.S.B.); Division of Cardiology (J.L.D.); Division of Endocrinology (B.D.); and Division of Endocrinology (J.E.B.R.), Veterans Administration Hospital, Aurora, Colorado 80045

Context: Cardiovascular disease is the major cause of death in adults with diabetes, yet little is specifically known about the effects of type 1 diabetes (T1D) on cardiovascular outcomes in youth. Although insulin resistance (IR) likely contributes to exercise and cardiovascular dysfunction in T2D, IR is not typically considered a contributor in T1D.

Objective: We hypothesized that cardiopulmonary fitness would be reduced in T1D youth in association with IR and cardiovascular dysfunction.

Design and Participants: This cross-sectional study at an academic hospital included 12 T1D adolescents compared with 12 nondiabetic controls, similar in age, pubertal stage, activity level, and body mass index.

Outcome Measures: Cardiopulmonary fitness was measured by peak oxygen consumption ($\text{VO}_{2\text{peak}}$) and oxygen uptake kinetics ($\text{VO}_{2\text{kinetics}}$), IR by hyperinsulinemic clamp, cardiac function by echocardiography, vascular function by venous occlusion plethysmography, intramyocellular lipid by magnetic resonance spectroscopy, and body composition by dual-energy x-ray absorptiometry.

Results: T1D adolescents had significantly decreased $\text{VO}_{2\text{peak}}$, peak work rate, and insulin sensitivity compared with nondiabetic adolescents. T1D youth also had reduced vascular reactivity and evidence of diastolic dysfunction and left ventricular hypertrophy. Despite their IR and reduced cardiovascular fitness, T1D youth had paradoxically normal intramyocellular lipid, waist to hip ratio, and serum lipids and high adiponectin levels. In multivariate analysis, IR primarily, and forearm blood flow secondarily, independently predicted $\text{VO}_{2\text{peak}}$.

Conclusions: T1D youth demonstrated IR, impaired functional exercise capacity and cardiovascular dysfunction. The phenotype of IR in T1D youth was unique, suggesting a pathophysiology that is different from T2D, yet may adversely affect long-term cardiovascular outcomes. (*J Clin Endocrinol Metab* 95: 513–521, 2010)

Cardiovascular disease (CVD) is the major cause of death in adults with diabetes mellitus, yet little is specifically known about the effects of type 1 diabetes mellitus (T1D) on cardiovascular outcomes in youth. T1D is characterized by absolute insulin deficiency from autoimmune destruction of pancreatic islet cells and typically presents in childhood and young adulthood. Despite evidence in the literature of insulin resistance (IR) in adults with T1D (1–3) and in adolescents during metabolic decompensation (4, 5) and puberty (6, 7), IR is not routinely considered to contribute to T1D pathophysiology.

Insulin sensitivity data are lacking in contemporary T1D youth. Such data are important, as the Neutral Protamine Hagedorn and regular insulin regimens in use at the time of the previously reported studies likely resulted in different glycemic profiles than are typical in children treated following the results of the Diabetes Control and Complications Trial, a study of youth and young adults with T1D that demonstrated the utility of tight glycemic control. Insulin sensitivity data are also lacking in T1D youth well controlled for age, pubertal stage, activity level, and body mass index (BMI), all of which likely influence insulin sensitivity.

We and others reported that cardiopulmonary fitness is reduced in adults and youth with type 2 diabetes (T2D) (8–10). Reduced cardiopulmonary fitness in T2D youth correlated strongly with IR (10). Some, although not all, studies examining exercise function in T1D exercise report abnormal peak oxygen uptake ($\text{VO}_{2\text{peak}}$) or peak workload in youth (11, 12) and adults (13, 14). One of these studies suggested an inverse relationship between fitness and IR (7).

Therefore, we hypothesized that cardiopulmonary fitness would be reduced in normal-weight adolescents with T1D, related to IR and markers of cardiovascular dysfunction. To test this hypothesis, we measured $\text{VO}_{2\text{peak}}$, oxygen uptake kinetics ($\text{VO}_{2\text{kinetics}}$) during submaximal constant-load exercise, and insulin sensitivity in normal-weight T1D adolescents as well as nondiabetic controls. Resting echocardiography and forearm reactive hyperemia were measured to assess possible cardiac and hemodynamic contributors to exercise dysfunction. Additionally, intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL), serum lipids and adiponectin, and body composition were measured to assess the potential relations of typical correlates of IR with the observed exercise responses.

Subjects and Methods

Subjects

Twenty-four adolescents were recruited from the University of Colorado Denver (UCD) pediatric diabetes clinics and by ad-

vertisement. Twelve T1D (50% female; one Black, one Hispanic, nine White, and one Other) and 12 healthy, nondiabetic controls (50% female; one Black, one Hispanic, nine White, and one Other) were identified of similar age, BMI, pubertal stage, gender, and habitual physical activity level. Mean age was 15.2 ± 2.2 yr (range 12–19 yr) and mean Tanner stage 4.5 ± 0.9 for females and 3.7 ± 0.9 for males.

The study was approved by the UCD Institutional Review Board, and appropriate consent was obtained. Changes in Colorado Institutional Review Board regulations limiting hyperinsulinemic clamps in control youth occurred after our previous adolescent T2D study. We therefore prospectively chose T1D adolescents to match the existing controls (10). T1D subjects were studied using identical methods.

Screening included a history, physical examination, Tanner staging, and fasting laboratory testing. T1D was defined by American Diabetes Association criteria plus the presence of glutamic acid decarboxylase, islet cell or insulin autoantibodies as well as insulin requirement. Absence of diabetes was confirmed in controls by a 2-h, 75-gram oral glucose tolerance test.

Inclusion criteria included Tanner stage higher than 1 and sedentary status (<3 h regular exercise/wk) to minimize pubertal and training effects. Exclusions included resting blood pressure higher than 140/90 mm Hg or higher than 190/100 mm Hg during exercise, hemoglobin lower than 9 mg/dl, serum creatinine higher than 1.5 mg/dl, glycosylated hemoglobin (HbA1c) higher than 11%, smoking, medication-dependent asthma, other conditions precluding exercise testing, medications affecting IR (oral or inhaled steroids, metformin, thiazolidinediones, or atypical antipsychotics), anti-hypertensive medications, oral contraceptives, pregnancy, breastfeeding, plans to alter exercise or diet during the study, and family history of T2D.

The insulin sensitivity of subjects in this study was also compared with the insulin sensitivity of age- and Tanner stage-matched obese (BMI $>95^{\text{th}}$ percentile) nondiabetic adolescents, and age-, Tanner stage-, and HbA1c-matched obese (BMI $>95^{\text{th}}$ percentile), diabetes autoantibody negative, non-insulin-requiring T2D adolescents we reported previously (10). These subjects were also similar in gender and habitual physical activity level and met the same inclusion and exclusion criteria with the exception of metformin use and family history of T2D in the subjects with T2D as the subjects reported here.

All subsequent tests were performed after a 12-h fast, preceded by 3 d restricted physical activity and a fixed-macronutrient, weight-maintenance diet (55% carbohydrates, 30% fat, 15% protein). T1D subjects were instructed to monitor blood glucose at least four times per day and were excluded if large urine ketones were present on admission.

Activity and diet questionnaires

A 3-d pediatric physical activity recall questionnaire was used to estimate habitual physical activity (15), reported as a 3-d average of daily metabolic equivalents. A food frequency questionnaire designed for youth with and without diabetes was used to estimate calorie and macronutrient intake (16).

Exercise testing

A graded cycle ergometer (Lode, Groningen, The Netherlands) protocol to exhaustion was used to determine $\text{VO}_{2\text{peak}}$, reported in milliliters per kilogram per minute (10). Perceived exertion was recorded every minute using the Borg scale (10).

Subjects were excluded if peak respiratory exchange ratio (RER) was less than 1.1.

Subjects also performed three identical cycle ergometer exercise bouts at a constant work rate equal to 85% of their estimated lactate threshold to determine VO_2 kinetics. VO_2 data were analyzed as previously reported (10). The phase 2 time constant of oxygen uptake is reported in seconds.

Blood sugars were closely monitored in T1D subjects, and short-acting insulin or carbohydrate given to achieve a goal pre-exercise range of 100–150 mg/dl.

Insulin sensitivity

Subcutaneous insulin was discontinued at dinner and replaced with an inpatient overnight iv insulin infusion to normalize blood sugar levels. Fasting blood and urine were collected for laboratory analyses, assayed by standard methods at the UCD research laboratory. A 3-h hyperinsulinemic-euglycemic clamp ($80 \text{ mU/m}^2 \cdot \text{min}$ insulin) was performed fasting from 0900–1200 h to estimate IR as previously described (10). Glucose disposal rate was expressed as milligrams per kilogram body mass and milligrams per kilogram fat-free mass.

Autonomic insufficiency

Variation in R-R intervals with cycled breathing, blood pressure, and heart rate responses to standing, and R-R variations with Valsalva breathing were measured (10). No subjects had autonomic dysfunction by these methods (data not shown).

Cardiovascular measurements

Resting two-dimensional and tissue Doppler echocardiography was performed using Vivid 7 and Echopac (BTO6 V6.1.3; General Electric, Milwaukee, WI) to exclude left ventricular systolic dysfunction (ejection fraction $<50\%$), regional wall motion abnormalities, pericardial disease, or significant valvular pathology. Electrocardiograms excluded resting ischemia. Left ventricular end-systolic and diastolic chamber dimensions and wall thickness, left atrial (LA) volumes (indexed for body surface area), fractional shortening, and biplane Simpson's method (chamber volumes) were quantitated by standard techniques. Velocity of propagation (Vp), mitral valve deceleration time, ratio of peak early mitral inflow velocity to peak late mitral inflow velocity (E/A), peak early mitral annular velocity (E/E'septal and E/E'lateral) and E/Vp were assessed by tissue Doppler for diastolic dysfunction (10).

Left ventricular hypertrophy was assessed by left ventricular posterior wall thickness in diastole (LVPWd), interventricular septal thickness in diastole (IVSd), left ventricular end-diastolic dimension (LVED), left ventricular end-systolic dimension (LVES), and left ventricular mass, indexed for height in meters (10, 17).

Forearm reactive hyperemic blood flow was determined by venous occlusion strain-gauge plethysmography (D. E. Hokanson, Issaquah, WA) using standard methods, expressed as peak hyperemic flow (milliliters per 100 ml per minute) (10).

Fat deposition

Body composition by dual-energy x-ray absorptiometry was performed by standard methods (18). IMCL and EMCL were measured via proton (^1H) magnetic resonance spectroscopy using 3.0 T whole-body magnetic resonance imaging (GE Medical Systems, Waukesha, WI). This technique exploits the small (~ 2

parts per million) frequency shift between IMCL and EMCL resonances observed when muscle fibers are roughly aligned in the direction of the magnetic field in a magnetic resonance scanner, as is the case with soleus and tibialis anterior muscles. Spectra were analyzed with LCModel software as previously described (10, 19). IMCL and EMCL concentrations, obtained by reference to the unsuppressed water peak, are reported in arbitrary concentration units.

Statistical analysis

Data are reported as mean \pm SD, and $\alpha < 0.05$ was considered statistically significant. Variances between groups were compared using the Lavine statistic and normality assessed by the Shapiro-Wilks test. *A priori* comparisons were made using ANOVA and *post hoc* analysis to correct for multiple comparisons (Bonferroni when variances were equal; Dunnett T3 when unequal). Planned univariate correlations between VO_2 peak and variables hypothesized to be causally associated were analyzed (Pearson correlation coefficients when data were normally distributed; Spearman's ρ when lacking normality) (SPSS version 13.0). Step-down multivariate linear regression analysis was performed including biologically plausible variables univariately correlated with VO_2 peak, with an α of ≤ 0.01 , to determine independent predictors (SAS version 9.1).

Results

Metabolic parameters (Table 1)

By design, groups were similar in age, Tanner stage, BMI, and gender. They were also similar in high-density

TABLE 1. Demographic characteristics

	Control	T1D
General		
n	12	12
Sex (% female)	50	50
Age (yr)	15.6 ± 1.8	14.8 ± 2.6
Tanner stage	4.4 ± 0.7	4.3 ± 0.8
BMI (kg/m^2)	21.0 ± 2.4	20.9 ± 3.1
Diabetes duration (yr)		7.5 ± 4
Lipids		
Cholesterol (mg/dl)	132 ± 22	163 ± 32^b
LDL cholesterol (mg/dl)	69.8 ± 19.0	94 ± 27.7^c
HDL cholesterol (mg/dl)	45.4 ± 8.4	48.5 ± 12.7
Triglycerides (mg/dl)	82 ± 37	86 ± 34
Triglyceride/HDL	1.93 ± 1.1	1.95 ± 1.1
Fasting free fatty acids ($\mu\text{Eq/liter}$)	491 ± 310	479 ± 204
Free fatty acids during insulin clamp ($\mu\text{Eq/liter}$)	48.9 ± 38.9	22.5 ± 16.6
Insulin sensitivity		
HbA1c (%)	4.9 ± 0.3	8.65 ± 1.6^a
Fasting C-peptide (ng/ml)	1.7 ± 0.6	0.025 ± 0.05^a
M ($\text{mg/kg} \cdot \text{min}$)	15.3 ± 4.7	9.6 ± 3.1^b
M ($\text{mg/lean kg} \cdot \text{min}$)	19.8 ± 4.6	13.3 ± 4.2^b

M, Glucose disposal rate.

^a $P < 0.001$ vs. control; ^b $P < 0.01$ vs. controls; ^c $P = 0.03$ vs. controls.

lipoprotein (HDL), blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, hemoglobin, hematocrit, platelets, urine microalbumin to creatinine ratio, and calorie and macronutrient intake, and no subjects had anemia (data not shown). T1D duration was 7.5 ± 4 yr (range 1–12 yr). T1D subjects had significantly higher fasting glucose (131.9 ± 46 mg/dl) and HbA1c and lower C-peptide than controls.

Fasting triglycerides, triglyceride to HDL ratio, free fatty acids, and free fatty acids drawn at 180 min of the hyperinsulinemic clamp were similar in T1D and controls. T1D subjects had significantly higher total and low-density lipoprotein (LDL) cholesterol *vs.* controls.

Exercise function (Table 2)

There were no significant differences between groups in habitual physical activity assessed by 3-d pediatric physical activity recall. Peak RER and heart rate during exercise testing were similar between groups, indicating similar maximal effort. VO_{2peak} (absolute VO_{2peak} , VO_{2peak}

per kilogram, and VO_{2peak} per kilogram fat-free mass) and peak work rate were significantly lower in T1D youth *vs.* controls.

Insulin sensitivity (Table 1)

Glucose disposal rates were significantly lower in T1D subjects *vs.* controls, whether expressed as milligrams per kilogram per minute or milligrams per kilogram lean body mass, consistent with significant IR. There were no significant differences in mean blood glucose or insulin levels between groups at the conclusion of the clamp. Glucose disposal rates did not correlate with HbA1c. Glucose disposal rates also did not correlate with insulin antibody levels, thus insulin antibodies did not appear to inhibit insulin action.

Cardiac and vascular function (Table 2)

Systolic and diastolic blood pressures were similar between groups. There were no abnormalities in fractional shortening, suggesting normal resting systolic function. T1D youth had significantly higher mitral valve E/A, E/E'septal, and E/Vp and significantly shorter Vp compared with controls, consistent with diastolic dysfunction. In addition, IVSd and indexed left ventricular mass were significantly greater in T1D subjects, indicating left ventricular hypertrophy. There were no significant differences in LA volume or LA volume indexed for body surface area between groups. However, indexed LA volume tended to be higher in T1D subjects, and four (22%) of the T1D subjects had an increased indexed LA volume (where normal is <30), whereas control subjects had normal values.

Peak forearm blood flow was significantly lower in T1D than control subjects and correlated inversely with LDL ($r = -0.4$; $P = 0.045$). No significant differences were noted in resting heart rate, recovery heart rate at 2 min, or rate of perceived exertion between groups during exercise testing (data not shown).

To determine whether diabetes duration mediated the cardiac abnormalities, an additional five T1D subjects with diabetes duration less than 3 yr (mean 1.5 yr) were recruited for echocardiograms alone. Subjects with longer duration had more evidence of left ventricular hypertrophy (IVSd 0.82 ± 0.11 cm in long duration *vs.* 0.68 ± 0.13 cm in shorter duration), but other echocardiographic parameters did not differ by diabetes duration. Data reported in Table 2 include all 17 T1D subjects, whose mean duration of diabetes was 6.1 ± 4 yr.

IVSd and LVPWd strongly correlated with lean body mass, as expected, and IVSd also correlated with diabetes duration ($r = 0.566$; $P = 0.014$). Mitral valve E:E'lateral correlated inversely with glucose disposal rate in milli-

TABLE 2. Exercise and cardiovascular function

	Control	T1D
Mean habitual activity (METs)	64 ± 11	64 ± 7
RER	1.18 ± 0.07	1.15 ± 0.05
Peak heart rate (beats/min)	184 ± 11	177 ± 15
VO_2 (ml/min)	2319 ± 530	1813 ± 416^b
VO_2/kg (ml/kg \cdot min)	40.4 ± 9.9	31.5 ± 7.6^d
$VO_2/lean$ kg (ml/lean kg \cdot min)	53.1 ± 8.5	42.9 ± 8.7^b
Peak work rate (watts)	220 ± 48.7	154 ± 32^b
τ_2 (sec)	28.6 ± 10	34.6 ± 8.7
Systolic blood pressure (mm Hg)	109 ± 6	106 ± 8
Diastolic blood pressure (mm Hg)	63 ± 6	66 ± 7
Peak forearm blood flow (ml/100 ml \cdot min)	17.6 ± 6.9	12.5 ± 5.6^e
FS (%)	35.1 ± 4.3	34 ± 4.9
MVDT (msec)	183 ± 47	213 ± 48^f
Vp (msec)	87 ± 19	57.9 ± 11^a
Mitral E/A	2.3 ± 0.4	2.9 ± 0.9^a
Mitral E/E'lateral	4.6 ± 0.9	4.9 ± 1.1
Mitral E/E'septal	6.1 ± 1.7	7.7 ± 1.8^c
E/Vp	1.20 ± 0.32	1.68 ± 0.53^a
IVSd (cm)	0.71 ± 0.12	0.76 ± 0.13
LVPWd (cm)	0.76 ± 0.10	0.78 ± 0.11
LVM (g)	100 ± 20.7	109 ± 30.1
Indexed LVM (g/m ^{2.7})	24 ± 4.3	28 ± 4.9^a
LA volume (ml)	38.9 ± 7.4	38.9 ± 12.4
Indexed LA volume [ml/(kg/m ^{2.7})]	22.9 ± 4.4	23.8 ± 5.6

τ_2 , Phase 2 time constant of oxygen uptake; A, peak late mitral inflow velocity; E, peak early mitral inflow velocity; E', peak early diastolic mitral annular velocity; FS, fractional shortening; LVM, left ventricular mass; LVPW, left ventricular Vp; METs, metabolic equivalents; MVDT, mitral valve deceleration time.

^a $P = 0.01$ *vs.* controls; ^b $P < 0.02$ *vs.* controls; ^c $P = 0.03$ *vs.* controls; ^d $P < 0.04$ *vs.* controls; ^e $P < 0.05$ *vs.* controls; ^f $P = 0.1$ *vs.* controls.

TABLE 3. Body composition and inflammation

	Control	T1D
Lean mass (kg)	44.8 ± 8.4	42.4 ± 6.5
Fat mass (kg)	11.01 ± 6.4	13.4 ± 5.9
% lean	79.4 ± 10.6	77.3 ± 6.6
% fat	20.4 ± 10.6	22.0 ± 6.6
Waist circumference (cm)	72.9 ± 7.5	69.8 ± 12
Hip circumference (cm)	83.2 ± 6.1	78.8 ± 15.1
Waist/hip	0.88 ± 0.06	0.89 ± 0.08
Soleus EMCL (IU)	1450 ± 863	1719 ± 1101
Tibialis EMCL (IU)	926 ± 723	1030 ± 489
Soleus IMCL (IU)	1225 ± 476	911 ± 300
Tibialis IMCL (IU)	373 ± 116	501 ± 295
Adiponectin (μg/ml)	8.4 ± 3.1	11.7 ± 8.8
WBC (10 ³ /μl)	6.0 ± 1.0	7.0 ± 1.8
hsCRP (mg/liter)	0.57 ± 0.3	0.9 ± 1.9
Myeloperoxidase (pmol/liter)	694 ± 396	612 ± 581
IL-6 (pg/ml)	1.2 ± 1.8	2.9 ± 6.7

WBC, White blood cells.

grams per kilogram per minute ($r = -0.62$; $P = 0.04$), and mitral E/A correlated significantly with HbA1c ($r = -0.47$; $P = 0.04$).

Fat deposition and inflammation (Table 3)

There were no significant differences between groups in fat-free mass or fat mass. T1D youth had high adiponectin levels and no significant differences in skeletal muscle IMCL, EMCL, or waist to hip ratio compared with controls. White blood cell count, highly sensitive C-reactive protein (hsCRP), IL-6, and myeloperoxidase were also similar between groups.

Correlates of VO₂peak

Insulin sensitivity (milligrams per kilogram per minute) was a significant predictor of VO₂peak ($r = 0.61$; $P = 0.007$) (Fig 1A) in a multivariate regression analysis controlled for age, Tanner stage, BMI, and habitual physical activity. Other *a priori* univariate correlates of VO₂peak included forearm blood flow ($r = 0.65$; $P = 0.001$) (Fig 1B), LDL ($r = -0.617$; $P = 0.003$), HbA1c ($r = -0.53$; $P = 0.007$), and mitral E/A ($r = -0.41$; $P < 0.05$). Fat distribution (waist to hip ratio or IMCL), triglyceride to HDL ratio, and inflammatory markers did not correlate with VO₂peak. Among T1D subjects alone, neither HbA1c nor diabetes duration correlated with VO₂peak. In a step-down multivariate linear regression analysis, only IR, forearm blood flow, and LDL remained as independent predictors of VO₂peak.

Discussion

This comprehensive investigation revealed several critical features of the effects of T1D on the cardiovascular system

in youth. These included impairment in functional exercise capacity and cardiac function in otherwise healthy, nonobese T1D youth compared with well-matched non-diabetic controls of similar BMI, pubertal stage, and baseline activity level. In addition, we found significant IR in these well-characterized, contemporary T1D youth, despite normal BMI, modern advances in insulin analogs, and lower glycemic targets. Finally, the T1D adolescents in this study manifested a unique phenotype of IR that included normal IMCL, body composition, waist to hip ratio, serum lipids, triglyceride to HDL ratio, and BMI as well as high adiponectin levels.

Of concern, these relatively well-controlled and normal-weight T1D adolescents had significant impairment in functional exercise capacity and cardiac and vascular function, even early in the T1D disease process. The reduced VO₂peak in T1D youth correlated with IR, similar to our findings in T2D youth (10), but not with other typically accepted manifestations of IR. In addition, HbA1c, a measure of glycemic control, correlated with VO₂peak only in univariate analysis, whereas IR, forearm blood flow, and LDL remained as independent predictors of VO₂peak in multivariate analysis, suggesting that exercise dysfunction is not primarily a function of poor glucose control in T1D. Mitral E/A as a measure of cardiac diastolic dysfunction also correlated with VO₂peak in univariate analysis. This cardiac parameter was not an independent predictor of VO₂peak, suggesting overlapping effects of IR, LDL, or vascular dysfunction on cardiac and exercise function. Although reported T1D studies vary regarding exercise function (11–14), our carefully controlled study, including control for baseline activity level, supports the majority of studies, which report exercise dysfunction in T1D.

T1D is well known to cause premature CVD, shortening average lifespan despite modern therapies (20). Unfortunately, the mechanisms underlying this association remain unclear. Classic CVD risk factors such as hypertension, dyslipidemia, and smoking are clearly important but do not completely explain the excess CVD risk in T1D (20) and were clearly not major contributors in the present study. The widespread existence of IR in T1D may provide a missing link, because IR and CVD are tightly connected in other populations (20). IR (estimated by an insulin sensitivity index), but not glycemia, predicted cardiovascular events and all-cause mortality in adults with childhood-onset T1D (21). Abnormal exercise capacity and its association with IR may also provide a clue, because poor fitness and CVD mortality are also closely linked.

Diabetes in adults is frequently associated with cardiac and vascular dysfunction, independent of coronary artery disease or hypertension (8, 9). Diastolic dysfunction is

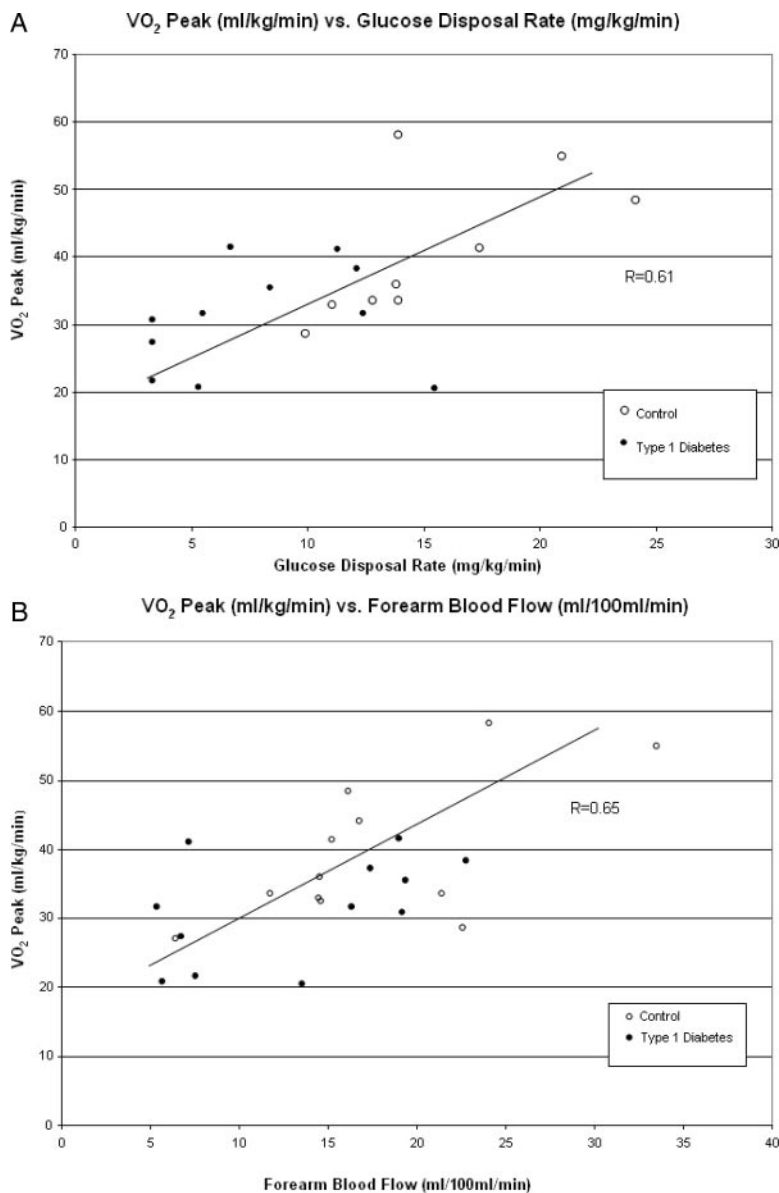


FIG. 1. A, Correlation between maximal exercise capacity (VO₂peak) expressed in milliliters per kilogram per minute and glucose disposal rate from the hyperinsulinemic-euglycemic clamp, expressed in milligrams per kilogram per minute; ○, control subjects; ●, T1D subjects. B, Correlation between maximal exercise capacity (VO₂peak) expressed in milliliters per kilogram per minute and peak hyperemic forearm blood flow from venous plethysmography, expressed in milliliters per 100 ml per minute; ○, control subjects; ●, T1D subjects.

reported twice as frequently as systolic dysfunction in T1D adults, with diastolic dysfunction occurring after approximately 8 yr of diabetes and systolic dysfunction after approximately 18 yr (22). In addition, diastolic dysfunction has been associated with enlarged LA dimension in subjects with diabetes (23, 24). Cardiac findings in T1D youth vary, with some but not all studies reporting abnormalities (11, 12, 25, 26). Confirming the findings of Suys *et al.* (26), our T1D adolescents had evidence of diastolic dysfunction and higher filling pressures at rest, despite only 6.1 yr of diabetes on average and no clinically evident CVD. We

also report evidence of enlarged atrial volumes in 22% of our T1D youth, which could decrease exercise capacity through pulmonary congestion. In the Suys study, HbA1c was similar to our study, and diabetes (not BMI or HbA1c) was the only important predictor in multiple linear regression models including control and diabetic subjects. Within diabetic subjects only, age was the only significant predictor. The multivariate model for contractility of Kimball *et al.* (25) included insulin dosage as a surrogate for IR, not HbA1c, and we similarly found that mitral valve E/E' lateral correlated inversely with glucose disposal rate, both studies suggesting that IR may affect cardiac function. IR could prevent the heart's adaptive switch from using predominately fat at rest to glucose during exercise, thereby decreasing performance (27). In addition, we did observe a significant correlation of mitral E/A with HbA1c that supports previous reports of negative impacts of hyperglycemia on cardiac function.

Forearm reactive blood flow by plethysmography was significantly reduced in our T1D adolescents, similar to our previous report in T2D youth (10). Flow-mediated dilation (FMD) has also been reported to be impaired in T1D youth of similar HbA1c (28, 29), suggesting endothelial dysfunction, one potential cause of the reduced forearm reactive blood flow we report. Vessel stiffness or reduced capillary density may also contribute to abnormal forearm reactive blood flow. Among T1D youth with impaired FMD studied by Järvisalo *et al.* (28), LDL but not HbA1c correlated with FMD, similar to our findings with plethysmography. Singh *et al.* (29) found lower FMD in diabetics, even after adjustment for glucose levels, only a weak correlation between FMD and HbA1c among all subjects, and a correlation with blood glucose levels and LDL, but not

HbA1c among diabetic subjects. These data further support the concept that factors beyond glycemic control, such as LDL cholesterol levels, may contribute to vascular dysfunction in T1D. Although mean LDL was not elevated by contemporary pediatric T1D standards, LDL was significantly higher in the T1D youth, which could directly interfere with endothelial function and limb hyperemia, and subsequently to reduced exercise capacity. Endothelial dysfunction could also contribute to IR, tying together our primary predictors of reduced exercise capacity.

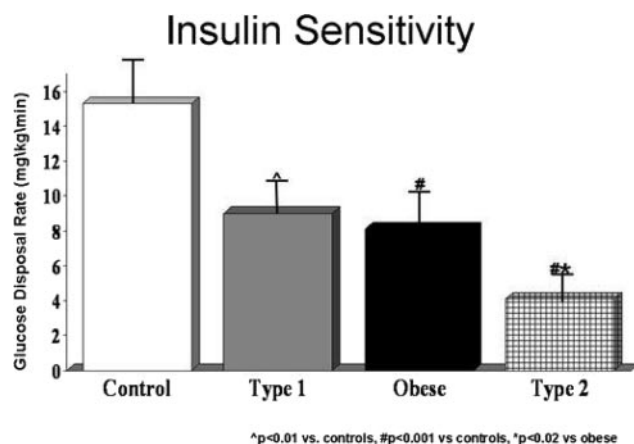


FIG. 2. Insulin sensitivity expressed as the glucose disposal rate in milligrams per kilogram per minute. Data from control (white bar), T1D (gray bar), obese (black bar), and type 2 diabetic (hatched bar) adolescents are displayed. ^ and #, *P* values compared with control adolescents; *, *P* values compared with obese adolescents.

IR has been appreciated in T1D adults (1–3) and adolescents during metabolic decompensation (4, 5) and historically in pubertal adolescents (6, 7) but not put into clinical context except in obese T1D patients. The mean HbA1c in our study (8.6%) was lower than in previous adolescent studies (ranging from mean HbA1c of 9.6–12.7%) (4–7), and Halldin *et al.* did not include nondiabetic controls. In addition, subjects in the studies from the 1980s used regular and lente or Neutral Protamine Hagedorn insulin, now rarely used in pediatrics. These regimens generally do not provide the degree of glycemic control currently attainable. Our data demonstrate continued IR in contemporary youth, despite normal BMI and modern advances in insulin analogs and lower glycemic targets, and are the first that compare T1D youth with nondiabetic controls well matched for BMI, body composition, Tanner stage, activity level, diet, and clamp insulin levels or relative to obese and T2D youth (10). Surprisingly, the degree of IR observed in T1D adolescents was similar to that of age- and Tanner stage-matched obese nondiabetic adolescents we reported previously (Fig 2), although not as impaired as HbA1c-matched obese T2D adolescents (10). This significant IR, along with our data showing the critical aspects of a unique IR phenotype and cardiovascular implications, needs to be considered early in T1D management.

Glycemic control in the adolescents we report is typical for current teenagers, although not optimal, and subjects with marked hyperglycemia were excluded. IR did not correlate with HbA1c, supporting the findings of Greenfield *et al.* (30) in adult females with T1D and the preliminary findings of Schauer *et al.* (31) in T1D adults, implying that factors beyond glycemia may contribute to the IR of T1D. However, some studies do show correlations be-

tween HbA1c and IR in T1D (1, 7), and elevated glucose levels in our subjects could still contribute to reduced skeletal muscle glucose transport (32) or hyperglycemia-induced abnormalities in muscle diacylglycerol kinase δ (33). Recent reports indicate additional mechanisms whereby glucose can mediate the IR of T1D, including O-glycosylation and altered phosphorylation of the insulin signaling pathway by O-linked beta N-acetylglucosamine and decreased insulin signal transduction (34, 35). Future studies will more closely examine the relationship between acute and chronic glycemia and IR in T1D and the impact of optimization of glucose control on the IR of T1D youth. Once established, the IR appears to have independent effects from glucose, because IR, not glycemia, remained as an independent predictor of VO_2peak .

Typically, IR is associated with a metabolic syndrome phenotype of high triglycerides, triglyceride to HDL ratio (36), visceral fat, IMCL, obesity, inflammation, and low HDL and adiponectin (37). In contrast, our T1D youth lacked these abnormalities. Single small studies individually found that T1D adults lacked central adiposity, hepatic steatosis, hypo adiponectinemia, or low HDL (30, 38, 39), supporting our observations. By showing all of these individually previously reported characteristics in combination, with the addition of normal IMCL, triglycerides, triglyceride to HDL ratio, BMI, and lack of inflammation, our data now comprehensively demonstrate that IR in T1D has a unique clinical phenotype.

Compensatory hyperinsulinemia typically contributes to the pathophysiology of IR, such as elevated IMCL. In our previous study of obese, IR T2D youth, IMCL was elevated and correlated inversely with VO_2peak (10), findings not observed in our T1D cohort. This difference could potentially be due to the absence of hyperinsulinemia. The lack of increased IMCL in T1D is supported by suggestions of normal IMCL in a small adult T1D study (40). In contrast, Perseghin *et al.* (1) reported increased IMCL in T1D adults. The latter subjects were older, with longer, more complicated T1D (retinopathy in 50%, sensorimotor neuropathy in 17%), which could limit the ability to exercise, a known regulator of IMCL.

Inflammation is typically a component of IR. Elevations of inflammatory cytokines have been reported in adults (41) and youth with T1D (42). We found no evidence of increased inflammation in this cohort of T1D youth, possibly because we excluded subjects with albuminuria (hsCRP and inflammation increase as microalbuminuria develops) (43).

Although we found no evidence of cardiac autonomic neuropathy in our subjects, such screening tests cannot completely exclude this as a contribution to exercise dysfunction, and cardiac autonomic neuropathy associated

with left ventricular diastolic dysfunction has previously been reported in adults with T1D and T2D (44, 45). Abnormal pulmonary function has also been documented in youth and adults with T1D (46–48), an additional factor not measured in this study that could contribute to exercise dysfunction.

Taken together, our observations present a distressing constellation of IR and exercise and cardiovascular dysfunction in normal-weight T1D children, despite short diabetes duration and absence of typical IR-related comorbidities. Unaddressed IR may contribute to the development of CVD in T1D. In addition, our data present a paradigm shift away from the traditional model of IR as being synonymous with the metabolic syndrome phenotype. We instead observed IR in T1D subjects without typical features of the metabolic syndrome, creating an entirely unique phenotype. Our data also suggest that IR may be a common, integral component of T1D pathophysiology. Elucidating the mechanisms underlying IR in adolescents with T1D will direct future research and therapeutic interventions. If IR in T1D is better understood and addressed early in the care of T1D patients, it may be possible to decrease cardiovascular morbidity and mortality in this population.

Acknowledgments

We thank our study participants for their time and efforts; Dr. Gerald Reaven for providing helpful feedback on this manuscript; and Stephen Belcher, Lindsay Ehlers, Leah Herliche, Lisa Herbert, Erik Sorenson, Rhonda Knapp-Clevenger, Ronald J. Sokol, and all of the Clinical and Translational Research Center staff for their excellent assistance; and Vermed, Inc., for providing the exercise electrodes.

Address all correspondence and requests for reprints to: Kristen J. Nadeau, M.D., Assistant Professor of Pediatrics, University of Colorado Health Sciences Center, The Children's Hospital, Department of Pediatric Endocrinology B265, 13123 East 16th Avenue, Aurora, Colorado 80045. E-mail: kristen.nadeau@ucdenver.edu.

K.J.N. is supported by a career development award from the National Institutes of Health (NIH)/National Center for Research Resources, 1 K23 RR020038-01. T.A.B. was supported by NIH Postdoctoral Fellowship Grant F32 DK078413-01. B.D. is supported by a Merit Review Award from the Department of Veterans Affairs. P.Z. is supported by a cooperative research project grant from the National Institute of Diabetes and Digestive and Kidney Diseases, NIH 5 U01 DK61242-02. J.E.B.R. is supported by Veterans Affairs Merit Review, NIH DK-64741, HL56481, and DK 57516. J.R. is supported by an American Diabetes Association Award. This research was also supported by Adult General Clinical Research Center NIH Grant M01-RR00051 and by Pediatric CTCRC NIH Grant 5M01 RR00069.

Disclosure Summary: The authors have nothing to disclose.

References

- Perseghin G, Lattuada G, Danna M, Sereni LP, Maffi P, De Cobelli F, Battezzati A, Secchi A, Del Maschio A, Luzi L 2003 Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab* 285: E1174–E1181
- DeFronzo RA, Hendler R, Simonson D 1982 Insulin resistance is a prominent feature of insulin-dependent diabetes. *Diabetes* 31:795–801
- Dabelea D, Kinney G, Snell-Bergeon JK, Hokanson JE, Eckel RH, Ehrlich J, Garg S, Hamman RF, Rewers M 2003 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
- Heptulla RA, Stewart A, Enocksson S, Rife F, Ma TY, Sherwin RS, Tamborlane WV, Caprio S 2003 In situ evidence that peripheral insulin resistance in adolescents with poorly controlled type 1 diabetes is associated with impaired suppression of lipolysis: a microdialysis study. *Pediatr Res* 53:830–835
- Halldin MU, Brismar K, Tuvemo T, Gustafsson J 2002 Insulin sensitivity and lipolysis in adolescent girls with poorly controlled type 1 diabetes: effect of anticholinergic treatment. *Clin Endocrinol (Oxf)* 57:735–743
- Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV 1986 Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 315:215–219
- Arslanian S, Nixon PA, Becker D, Drash AL 1990 Impact of physical fitness and glycemic control on in vivo insulin action in adolescents with IDDM. *Diabetes Care* 13:9–15
- Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR 1995 Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Med Sci Sports Exerc* 27:875–881
- Poirier P, Garneau C, Bogaty P, Nadeau A, Marois L, Brochu C, Gingras C, Fortin C, Jobin J, Dumesnil JG 2000 Impact of left ventricular diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 diabetes mellitus. *Am J Cardiol* 85:473–477
- Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, Regensteiner JG, Reusch JEB 2009 Insulin Resistance in Adolescents with Type 2 Diabetes is Associated with Impaired Exercise Capacity. *J Clin Endocrinol Metab* epub ahead of print
- Rowland TW, Martha Jr PM, Reiter EO, Cunningham LN 1992 The influence of diabetes mellitus on cardiovascular function in children and adolescents. *Int J Sports Med* 13:431–435
- Gusso S, Hofman P, Lalonde S, Cutfield W, Robinson E, Baldi JC 2008 Impaired stroke volume and aerobic capacity in female adolescents with type 1 and type 2 diabetes mellitus. *Diabetologia* 51: 1317–1320
- Wanke T, Formanek D, Auinger M, Zwick H, Irsigler K 1992 Pulmonary gas exchange and oxygen uptake during exercise in patients with type 1 diabetes mellitus. *Diabet Med* 9:252–257
- Niranjan V, McBrayer DG, Ramirez LC, Raskin P, Hsia CC 1997 Glycemic control and cardiopulmonary function in patients with insulin-dependent diabetes mellitus. *Am J Med* 103:504–513
- Weston AT, Petosa R, Pate RR 1997 Validation of an instrument for measurement of physical activity in youth. *Med Sci Sports Exerc* 29:138–143
- Mayer-Davis EJ, Vitolins MZ, Carmichael SL, Hemphill S, Tsaroucha G, Rushing J, Levin S 1999 Validity and reproducibility of a food frequency interview in a multi-cultural epidemiology study. *Ann Epidemiol* 9:314–324
- de Simone G, Daniels SR, Devereux RB, Meyer RA, Roman MJ, de Divitiis O, Alderman MH 1992 Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. *J Am Coll Cardiol* 20:1251–1260
- Kamel EG, McNeill G, Han TS, Smith FW, Avenell A, Davidson L, Tothill P 1999 Measurement of abdominal fat by magnetic reso-

- nance imaging, dual-energy x-ray absorptiometry and anthropometry in non-obese men and women. *Int J Obes Relat Metab Disord* 23:686–692
19. Thamer C, Machann J, Bachmann O, Haap M, Dahl D, Wietek B, Tschritter O, Niess A, Brechtel K, Fritsche A, Claussen C, Jacob S, Schick F, Haring H, Stumvoll M 2003 Intramyocellular lipids: anthropometric determinants and relationships with maximal aerobic capacity and insulin sensitivity. *J Clin Endocrinol Metab* 88:1785–1791
 20. Libby P, Nathan DM, Abraham K, Brunzell JD, Fradkin JE, Haffner SM, Hsueh W, Rewers M, Roberts BT, Savage PJ, Skarlatos S, Wassef M, Rabadan-Diehl C 2005 Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Disease Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 111:3489–3493
 21. Orchard TJ, Olson JC, Erbey JR, Williams K, Forrest KY, Smithline Kinder L, Ellis D, Becker DJ 2003 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
 22. Raev DC 1994 Which left ventricular function is impaired earlier in the evolution of diabetic cardiomyopathy? An echocardiographic study of young type I diabetic patients. *Diabetes Care* 17:633–639
 23. Park JW, Ziegler AG, Janka HU, Doering W, Mehnert H 1988 Left ventricular relaxation and filling pattern in diabetic heart muscle disease: an echocardiographic study. *Klin Wochenschr* 66:773–778
 24. Muranaka A, Yuda S, Tsuchihashi K, Hashimoto A, Nakata T, Miura T, Suzuki M, Wakabayashi C, Watanabe N, Shimamoto K 2009 Quantitative assessment of left ventricular and left atrial functions by strain rate imaging in diabetic patients with and without hypertension. *Echocardiography* 26:262–271
 25. Kimball TR, Daniels SR, Khoury PR, Magnotti RA, Turner AM, Dolan LM 1994 Cardiovascular status in young patients with insulin-dependent diabetes mellitus. *Circulation* 90:357–361
 26. Suys BE, Katier N, Rooman RP, Matthys D, Op De Beeck L, Du Caju MV, De Wolf D 2004 Female children and adolescents with type 1 diabetes have more pronounced early echocardiographic signs of diabetic cardiomyopathy. *Diabetes Care* 27:1947–1953
 27. Regensteiner JG, Bauer TA, Reusch JE, Brandenburg SL, Sippel JM, Vogelsson AM, Smith S, Wolfel EE, Eckel RH, Hiatt WR 1998 Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. *J Appl Physiol* 85:310–317
 28. Järvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT 2004 Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation* 109:1750–1755
 29. Singh TP, Groehn H, Kazmers A 2003 Vascular function and carotid intimal-medial thickness in children with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 41:661–665
 30. Greenfield JR, Samaras K, Chisholm DJ 2002 Insulin resistance, intra-abdominal fat, cardiovascular risk factors, and androgens in healthy young women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 87:1036–1040
 31. Schauer IE, Snell-Bergeon JK, Bergman BC, Maahs D, Eckel RH, Rewers M 2009 Sex-based differences in type 1 diabetes-associated insulin resistance. *Diabetes* 58:A32
 32. Koivisto VA, Yki-Järvinen H 1990 Changes in muscle glucose metabolism in type 1 diabetes. *Ann Med* 22:201–205
 33. Chibalin AV, Leng Y, Vieira E, Krook A, Björnholm M, Long YC, Kotova O, Zhong Z, Sakane F, Steiler T, Nylén C, Wang J, Laakso M, Topham MK, Gilbert M, Wallberg-Henriksson H, Zierath JR 2008 Downregulation of diacylglycerol kinase delta contributes to hyperglycemia-induced insulin resistance. *Cell* 132:375–386
 34. Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, So WV, Kudlow JE, Michell RH, Olefsky JM, Field SJ, Evans RM 2008 Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. *Nature* 451:964–969
 35. Dentin R, Hedrick S, Xie J, Yates 3rd J, Montminy M 2008 Hepatic glucose sensing via the CREB coactivator CRTC2. *Science* 319:1402–1405
 36. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G 2003 Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 139:802–809
 37. Cali AM, Caprio S 2008 Obesity in children and adolescents. *J Clin Endocrinol Metab* 93:S31–S36
 38. Perseghin G, Lattuada G, De Cobelli F, Esposito A, Costantino F, Canu T, Scifo P, De Taddeo F, Maffi P, Secchi A, Del Maschio A, Luzzi L 2005 Reduced intrahepatic fat content is associated with increased whole-body lipid oxidation in patients with type 1 diabetes. *Diabetologia* 48:2615–2621
 39. Taskinen MR 1992 Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. *Diabetes* 41(Suppl 2):12–17
 40. Bernroider E, Brehm A, Krssak M, Anderwald C, Trajanoski Z, Cline G, Shulman G, Roden M 2005 The role of intramyocellular lipids during hypoglycemia in patients with intensively treated type 1 diabetes. *J Clin Endocrinol Metab* 90:5559–5565
 41. Devaraj S, Cheung AT, Jialal I, Griffen SC, Nguyen D, Glaser N, Aoki T 2007 Evidence of increased inflammation and microcirculatory abnormalities in patients with type 1 diabetes and their role in microvascular complications. *Diabetes* 56:2790–2796
 42. Rosa JS, Flores RL, Oliver SR, Pontello AM, Zaldivar FP, Galassetti PR 2008 Sustained IL-1 α , IL-4, and IL-6 elevations following correction of hyperglycemia in children with type 1 diabetes mellitus. *Pediatr Diabetes* 9:9–16
 43. Marcovecchio ML, Giannini C, Widmer B, Dalton RN, Martinotti S, Chiarelli F, Dunger DB 2008 C-reactive protein in relation to the development of microalbuminuria in type 1 diabetes: the Oxford Regional Prospective Study. *Diabetes Care* 31:974–976
 44. Poirier P, Bogaty P, Philippon F, Garneau C, Fortin C, Dumesnil JG 2003 Preclinical diabetic cardiomyopathy: relation of left ventricular diastolic dysfunction to cardiac autonomic neuropathy in men with uncomplicated well-controlled type 2 diabetes. *Metabolism* 52:1056–1061
 45. Karavanaki K, Kazianis G, Konstantopoulos I, Tsouvalas E, Karayianni C 2008 Early signs of left ventricular dysfunction in adolescents with type 1 diabetes mellitus: the importance of impaired circadian modulation of blood pressure and heart rate. *J Endocrinol Invest* 31:289–296
 46. Cazzato S, Bernardi F, Salardi S, Tassinari D, Corsini I, Ragni L, Cicognani A, Cacciari E 2004 Lung function in children with diabetes mellitus. *Pediatr Pulmonol* 37:17–23
 47. Boulbou MS, Gourgoulanis KI, Klisiaris VK, Tsikrikas TS, Stathakis NE, Molyvdas PA 2003 Diabetes mellitus and lung function. *Med Princ Pract* 12:87–91
 48. Schuyler MR, Niewoehner DE, Inkley SR, Kohn R 1976 Abnormal lung elasticity in juvenile diabetes mellitus. *Am Rev Respir Dis* 113:37–41