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Vascular, metabolic, and inflammatory abnormalities in normoglycemic offspring of patients with type 2 diabetes mellitus

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Abstract

Endothelial dysfunction, insulin resistance, and elevated levels of circulating proinflammatory markers are among the earliest detectable abnormalities in people at risk for atherosclerosis. Accelerated atherosclerosis is a leading contributor to morbidity and mortality in type 2 diabetes mellitus, a complex genetic disorder. Therefore, we hypothesized that normoglycemic offspring of patients with type 2 diabetes mellitus (NOPD) may have impaired vascular and metabolic function related to an enhanced proinflammatory state. We compared NOPD (n = 51) with matched healthy control subjects without family history of diabetes (n = 35). Flow- and nitroglycerin-mediated brachial artery vasodilation were assessed by ultrasound to evaluate endothelium-dependent and -independent vascular function. Each subject also underwent an oral glucose tolerance test to evaluate metabolic function. Fasting levels of plasma adiponectin and circulating markers of inflammation (high-sensitivity C-reactive protein, CD40 ligand, interleukin 1β, tumor necrosis factor α, vascular cell adhesion molecule 1, and intracellular adhesion molecule) were measured. Both NOPD and the control group had fasting glucose and insulin levels well within the reference range. However, results from oral glucose tolerance test and quantitative insulin sensitivity check index revealed that NOPD were insulin resistant with significantly impaired flow- and nitroglycerin-mediated dilation compared with the control group. Adiponectin levels were lower, whereas many circulating markers of inflammation were higher, in NOPD compared with the control group. Normoglycemic offspring of patients with type 2 diabetes mellitus have impaired vascular and metabolic function accompanied by an enhanced proinflammatory state that may contribute to their increased risk of diabetes and its vascular complications.
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1. Introduction

Atherosclerotic vascular disease is a leading cause of morbidity and mortality in patients with type 2 diabetes mellitus [1]. Similar genetic and environmental factors may contribute independently to both atherosclerosis and type 2 diabetes mellitus [2]. Endothelial dysfunction, characterized by reduced nitric oxide (NO)–dependent vascular activity and a reciprocal relationship with insulin resistance [2], is one of the earliest detectable features in the development of atherosclerosis and coronary heart disease [3]. Strikingly, approximately 50% of diabetic patients already have established coronary heart disease at the time of diagnosis [4]. Thus, increased prevalence of cardiovascular disease precedes development of clinically overt diabetes. In addition to NO-dependent endothelial dysfunction, patients with type 2 diabetes mellitus have impaired vascular responses to exogenous NO donors [5] and enhanced responsiveness to vasoconstrictor agents [6,7]. In normoglycemic offspring of patients with type 2 diabetes mellitus (NOPD), significant endothelial dysfunction and insulin

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resistance is detectable even in the absence of frank diabetes [8,9]. This suggests that genetic factors contributing to insulin resistance and diabetes may also influence development of cardiovascular diseases including atherosclerosis and coronary heart disease that are related to endothelial dysfunction.

Proinflammatory cytokines may play important roles in the pathogenesis of both endothelial dysfunction and insulin resistance [2]. In some epidemiologic studies, elevated plasma levels of acute phase proteins including high-sensitivity C-reactive protein (hsCRP) predict the onset of both type 2 diabetes mellitus [10] and cardiovascular disease [11] in otherwise healthy subjects. Similarly, elevated levels of CD40 ligand (CD40L) prospectively predict cardiovascular events in otherwise healthy women [12] and elevated soluble CD40L levels are present in patients with diabetes [13]. CD40 signaling is known to mediate many inflammatory responses in atherosclerosis [14]. Tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), proinflammatory cytokines implicated in the pathogenesis of insulin resistance [15], may also contribute to progression of atherosclerosis [16]. Interestingly, it is estimated that genetic factors constitute approximately 40% of the determinants of baseline values of hsCRP, whereas low-grade chronic inflammatory status may be determined by genetic factors in addition to environmental stressors [17,18]. Taken together, these findings support a common role for inflammation in the development of both diabetes and atherosclerosis that may have significant genetic determinants. To gain further insight into relationships among genetics, insulin resistance, endothelial dysfunction, and inflammation, in the present study we evaluated vascular function, metabolic parameters, and circulating inflammatory markers in healthy offspring of patients with type 2 diabetes mellitus and matched control subjects without diabetic parents.

2. Subjects, materials, and methods

2.1. Study design and study subjects

We evaluated vascular and metabolic function as well as inflammatory markers in healthy Italian subjects who had at least one parent with type 2 diabetes mellitus. We compared these results with those obtained in a matched, healthy control group who did not have parents with diabetes. Fifty-one subjects who were offspring of diabetic parents identified in the Clinical Center for Atherosclerosis at the University of Rome, Tor Vergata, were enrolled and completed all phases of the study. Thirty-five healthy subjects matched for sex, age, body mass index (BMI), and blood pressure, with no history of type 2 diabetes mellitus in any first-degree relatives, were enrolled as a control group. All participating women reported regular menstrual cycles and none of them were receiving oral contraceptives. In these women, endothelial function testing and oral glucose tolerance test (OGTT) were performed during the first week of the menstrual cycle. None of the study participants were taking any medication, including aspirin or vitamin supplements, at the time of the study. Before enrollment, subjects were screened by clinical history, physical examination, electrocardiography, chest x-ray, and routine chemical analyses. Exclusion criteria were history or evidence of hypertension (blood pressure >140/90 mm Hg), diabetes mellitus according to the American Diabetes Association criteria [19], cardiac disease, peripheral vascular disease, coagulopathy, or any other disease predisposing to vasculitis or Raynaud phenomenon. The study protocol was approved by the University of Tor Vergata Institutional Review Board and all participants gave written informed consent for all procedures.

2.2. Vascular function studies

All studies were performed in the morning in a quiet room with a temperature of about 22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine for at least 24 hours before the study, and all study subjects fasted for at least 10 hours before the study day. Endothelium-dependent and -independent vasodilator functions were assessed following currently published guidelines [20]. Briefly, subjects lay supine on a bed and were allowed to rest for at least 10 minutes. Then, the left brachial artery was visualized 2 to 15 cm proximal to the antecubital fossa by using a high-resolution ultrasound (ATL HDI 3000, with a 7.5-MHz linear array transducer, Philips Medical Systems, Best, the Netherlands). After baseline images and flow measurements were obtained, a pressure cuff applied on the upper arm was inflated at 200 to 250 mm Hg for 5 minutes. Blood flow was measured during the 15 seconds following cuff release, and arterial images for diameter measurement were acquired between 60 and 90 seconds after cuff deflation. Flow-mediated dilation (FMD) was calculated as the increase in poststimulus diameter as a percentage of the baseline diameter. After at least 15 minutes rest, endothelium-independent vasomotor responsiveness was assessed by acquiring images and flow measurements before and after 0.4 mg of sublingual nitroglycerin was given. Blood flow and images for arterial diameter were recorded between 3 and 4 minutes after nitroglycerin administration. Nitroglycerin-mediated dilation (NMD) was calculated as the increase in poststimulus diameter as a percentage of the baseline diameter. For both FMD and NMD, arterial diameter was measured from the anterior to the posterior endothelial-lumen interface at end diastole, coincident with the R wave on the electrocardiogram. Images were then coded and analyzed by an investigator blinded to image sequence and subject group.

2.3. Metabolic function studies

At the beginning of each study day, fasting blood samples were taken to evaluate lipid profiles, glycosylated hemoglobin (HbA_{1c}), plasma adiponectin, and routine

hematologic and biochemical analytes. After completion of the vascular function studies, each subject then underwent a standard OGTT. Seventy-five grams glucose was given orally and venous blood samples were drawn at 0, 30, 60, 90, and 120 minutes after glucose ingestion for determination of blood glucose and plasma insulin concentrations. Surrogate indexes of insulin sensitivity (quantitative insulin sensitivity check index [QUICKI] [21], Matsuda index [22]) and insulin resistance (log homeostasis model assessment [HOMA] [23]) were calculated as described.

2.4. Circulating inflammatory markers

At the beginning of each study day, fasting blood samples were taken to evaluate hsCRP, CD40L, TNF-α, IL-1β, vascular cell adhesion molecule 1 (VCAM-1), and intracellular adhesion molecule (ICAM) by using commercially available kits. Serum hsCRP levels were measured by a nephelometric assay (Dade-Behring, Liederbach, Germany). CD40L, IL-1β, VCAM-1, ICAM, and TNF-α levels were evaluated by enzyme-linked immunosorbent assay (Bender MedSystem, Vienna, Austria). For TNF-α, IL-1β, VCAM-1, and ICAM, levels were not obtained from all study subjects because the amount of blood obtained from some study subjects was not adequate.

2.5. Statistical analysis

The primary outcome measure for this study was prospectively designated as the difference in FMD between offspring of diabetic parents and control subjects. Power calculations indicated that a sample size of 35 was sufficient to detect a 2.5% difference in FMD, with 80% power and an α of .05, assuming that the variance in FMD in our subjects is similar to that in middle-aged men reported by Vogel et al [24]. Group differences were analyzed by unpaired Student *t* test or the Mann-Whitney test for parametric and nonparametric data, respectively. Differences in glucose and insulin values during OGTT were evaluated by 2-way analysis of variance. Values of $P < .05$ were considered to indicate statistical significance. Statistical analyses were performed using SigmaStat 3.1 (Systat Software, Point Richmond, CA).

Table 1
Anthropometric and biochemical characteristics of study groups

	Nondiabetic offspring of diabetic Parents (n = 51)	Healthy subjects without diabetic parents (n = 35)	*P*
Age (y)	31 ± 1	29 ± 1	.10
Sex (female/male)	23/28	24/11	
Smokers/Nonsmokers	9/42	5/30	
BMI (kg/m2)	24 ± 1	23 ± 1	.10
Waist (cm)	84 ± 2	80 ± 2	.11
SBP (mm Hg)	113 ± 2	113 ± 2	.70
DBP (mm Hg)	73 ± 1	75 ± 1	.20
MAP (mm Hg)	86 ± 1	85 ± 3	.60
Total cholesterol (mg/dL)	182 ± 5	172 ± 4	.14
LDL cholesterol (mg/dL)	116 ± 4	105 ± 4	.10
HDL Cholesterol (mg/dL)	61 ± 2	67 ± 3	.03
TG (mg/dL)	90 ± 5	69 ± 5	.03
HbA_{1c} (%)	5.2 ± 0.03	5.1 ± 0.03	.07
VCAM-1 (ng/mL)	970 ± 44 (n = 20)	987 ± 63 (n = 14)	.89
ICAM-1 (ng/mL)	261 ± 10 (n = 20)	279 ± 9 (n = 14)	.60

Data are expressed as mean ± SEM of n subjects in each group. Statistical comparisons were made using Student *t* test and Mann-Whitney test for parametric and nonparametric data, respectively. VCAM-1 and ICAM-1 measurements were obtained from only a subset of subjects as indicated. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides.

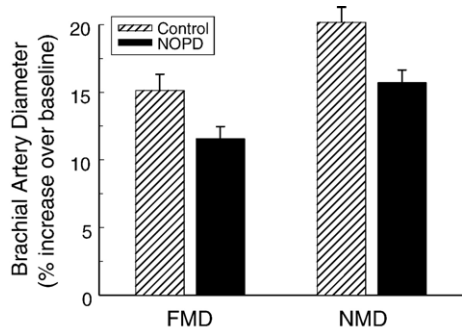

Fig. 1. Endothelial- and non–endothelial-dependent vasodilation in the brachial artery are both significantly impaired in NOPD (black bars) compared with healthy control subjects (Control, hatched bars) ($P < .02$). FMD reflects endothelial-dependent vasodilation, whereas NMD reflects non–endothelial-dependent vasodilation. Data are mean ± SEM expressed as percent increase over baseline.

3. Results

3.1. Study subjects

Anthropometric and biochemical characteristics of 51 NOPD and 35 matched healthy control subjects without diabetic parents are reported in Table 1. Both groups were healthy with normal BMI, blood pressure, lipids, and HbA_{1c}. Moreover, the study group and control group were similar in age, sex, smoking history, BMI, blood pressure, total cholesterol, and low-density lipoprotein cholesterol. Triglyceride levels were higher in the study group, whereas high-density lipoprotein cholesterol levels were lower in the study group compared with the control group. VCAM-1 and ICAM levels measured in 20 study subjects and 14 controls were not statistically different.

3.2. Vascular function studies

Brachial artery diameter was assessed before and after reactive hyperemia or sublingual nitroglycerin to evaluate FMD and NMD in the NOPD group as well as in control subjects (Fig. 1). Baseline brachial artery diameter before hyperemia was similar in the NOPD group and controls (3.3 ± 0.08 vs 3.0 ± 0.08 mm, respectively). Mean reactive hyperemia after cuff deflation was also similar between NOPD and control groups (1147% ± 160% vs 707% ± 87%, $P > .3$). Mean postreactive hyperemia brachial artery

diameter was 3.7 ± 0.06 and 3.5 ± 0.01 mm in the NOPD and control group, respectively; the mean diameter after nitroglycerin administration was 3.9 ± 0.06 and 3.4 ± 0.01 mm in the NOPD and control group, respectively. Of note, FMD (percent increase over baseline) was significantly lower in the NOPD group compared with the control group (11.5% ± 0.8% vs 15.1% ± 1.2%, $P < .02$). Similarly, NMD (percent increase over baseline) was significantly lower in the NOPD group compared with the control group (15.7% ± 0.8% vs 20.1% ± 1.1%, $P < .01$). Thus, both endothelial-dependent and -independent vascular functions are impaired in the NOPD group compared with matched controls.

3.3. Metabolic studies

Because there is a reciprocal relationship between endothelial dysfunction and insulin resistance [2], we also evaluated metabolic parameters in our study subjects. Both NOPD and control groups had normal fasting glucose and insulin levels. However, fasting glucose levels were significantly higher in the NOPD group compared with the control group (Table 2). Fasting insulin levels tended to be higher in the NOPD group than in the control group, although this difference did not reach statistical significance ($P < .11$). More importantly, 3 surrogate indexes of insulin sensitivity or insulin resistance (QUICKI, Matsuda index, and log HOMA) indicated that the NOPD group had decreased insulin sensitivity compared with the control group (Table 2). Results from OGTT were consistent with the presence of decreased insulin sensitivity in the first-degree group (Fig. 2A and B). The blood glucose profile during OGTT in the NOPD group was significantly higher than that in the control group although the insulin profile was also higher in the NOPD group than in the control group. That is, even with a more robust insulin response to oral glucose challenge, the glucose tolerance of the NOPD group was impaired relative to the control group. Thus, both fasting and dynamic assessments indicate impairment of insulin sensitivity in the NOPD group relative to the control group. Adiponectin is a hormone secreted exclusively by adipose cells whose levels are positively correlated with insulin sensitivity [15]. Fasting plasma adiponectin levels in the NOPD group were significantly decreased compared with the control group (Fig. 2C). These results are also consistent with the relative decrease in insulin sensitivity observed in the NOPD group.

Table 2
Metabolic parameters of study groups

	Nondiabetic offspring of diabetic parents (n = 51)	Healthy subjects without diabetic parents (n = 35)	P
Fasting glucose (mg/dL)	88 ± 1	84 ± 1	.03
Fasting insulin (μU/mL)	8.5 ± 0.5	7.0 ± 0.4	.11
QUICKI	0.356 ± 0.003	0.366 ± 0.004	.04
Matsuda index	109 ± 7	138 ± 10	.02
Log HOMA	0.225 ± 0.03	0.134 ± 0.03	.03

Data are expressed as mean ± SEM of n subjects in each group. Statistical comparisons were made using Student *t* test and Mann-Whitney test for parametric and nonparametric data, respectively. Surrogate indexes for insulin sensitivity and resistance were calculated as described in "Subjects, materials, and methods".

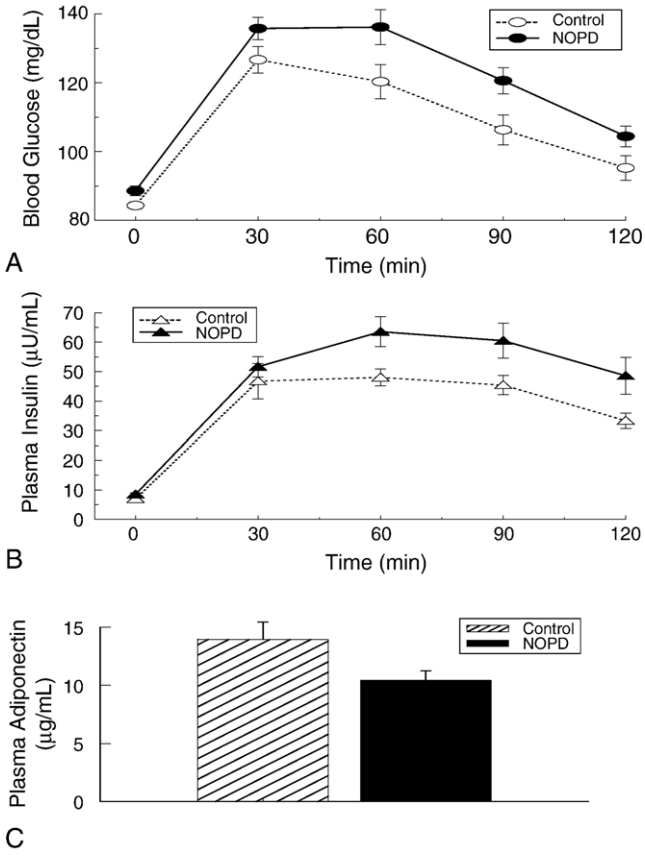

Fig. 2. OGTT results and plasma adiponectin levels in NOPD (black symbols and bars) are consistent with increased insulin resistance compared with healthy control subjects (Control, open symbols and hatched bars). Blood glucose levels (A) and plasma insulin levels (B) during OGTT, and fasting plasma adiponectin levels (C) are plotted as mean ± SEM. Blood glucose and plasma insulin levels during OGTT are significantly higher in the NOPD group compared with the control group ($P < .001$ by 2-way analysis of variance). Fasting plasma adiponectin levels are significantly lower in the NOPD group compared with the control group ($P < .04$).

3.4. Circulating markers of inflammation

Proinflammatory states have been implicated in the development of both endothelial dysfunction and insulin resistance [2]. Therefore, we also evaluated a variety of circulating markers of inflammation (Fig. 3). CRP and CD40L were significantly elevated in the NOPD group compared with the control group (Fig. 3A and B). Moreover, TNF-α levels were also elevated when 26 NOPD subjects were compared with 26 controls (Fig. 3C). Similarly, IL-1β levels were elevated in 48 NOPD group subjects compared with 32 controls (Fig. 3D). Thus, consistent with the impaired vascular function and relative decrease in insulin sensitivity, circulating markers of inflammation were elevated in NOPD subjects.

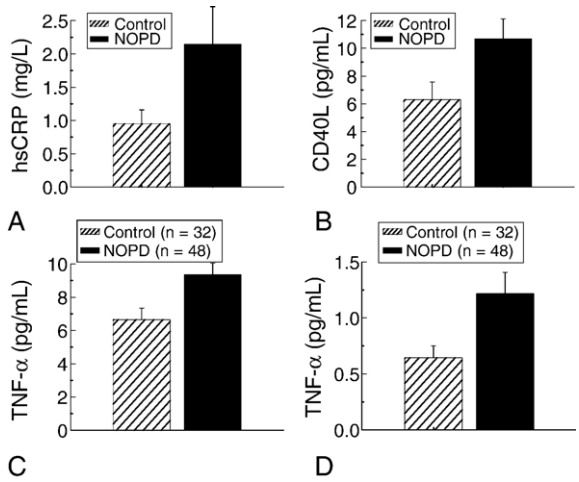

Fig. 3. Circulating markers of inflammation are elevated in NOPD (black bars) compared with healthy control subjects (Control, hatched bars). Plasma concentrations of hsCRP (A), CD40L (B), TNF-α (C), and IL-1β (D) shown are the mean ± SEM. For the inflammatory markers shown, the NOPD group had significantly higher levels than the control group ($P < .02$).

3.5. Correlations among study parameters

To more fully characterize relationships among metabolic variables, inflammatory markers, and vascular responsiveness in the NOPD group, stepwise regression analyses were performed. Stepwise linear regression yielded a model in which adiponectin was a significant predictor of FMD ($r = 0.532$, $P = .006$), independent of CRP, TNF-α levels, and area under the curve insulin values from OGTT.

4. Discussion

Diabetes, coronary heart disease, and atherosclerosis are major interrelated public health problems that all have strong genetic components [1,3]. An enhanced proinflammatory state has been implicated in the pathogenesis of all of these diseases. Indeed, proinflammatory cytokines may provide one link underlying reciprocal relationships between insulin resistance and endothelial dysfunction [2]. Therefore, it is of interest to determine whether early and related abnormalities in vascular function, metabolic status, and circulating markers of inflammation can be identified in seemingly healthy normoglycemic offspring of diabetic patients who may be at greater risk for serious health problems than the general population.

4.1. Metabolic outcomes

In our study in an Italian cohort, the NOPD were seemingly healthy with normal BMI, blood pressure, total cholesterol, and HbA_{1C} compared with matched healthy controls without diabetic parents. However, in the NOPD group, fasting triglyceride levels were modestly elevated, plasma adiponectin levels were decreased, and results from both OGTT and 3 different surrogate indexes of insulin sensitivity/resistance indicated decreased insulin sensitivity in the NOPD group compared with the control group. This suggests that the NOPD group was more insulin resistant on average than the control group. Thus, our data support the presence of an important genetic component underlying insulin resistance that may contribute to the development of diabetes. This is not surprising because other studies have documented insulin resistance and decreased adiponectin levels in nondiabetic offspring of diabetic parents [25,26].

4.2. Vascular outcomes

The primary end point for the present study was to evaluate differences in FMD between the NOPD and control groups. Both baseline brachial artery diameter and reactive hyperemia after cuff deflation were similar between NOPD and control groups. However, both FMD and NMD were significantly lower in the NOPD group compared with the control group. This suggests that in addition to decreased insulin sensitivity, otherwise healthy subjects with a family history of type 2 diabetes mellitus have impairment in vascular responsiveness to NO that may be endothelium dependent and/or independent. It is well established that patients with diabetes have endothelial dysfunction. In particular, patients with type 2 diabetes mellitus have blunted reactivity to both metacholine (a measure of NO-mediated endothelium-dependent vasodilation) and sodium nitroprusside (an endothelium-independent NO donor) [5]. Thus, our data on endothelial function in NOPD suggest that, in addition to insulin resistance, endothelial dysfunction may have genetic determinants that help to link cardiovascular and metabolic diseases. Alternatively, or in addition, reciprocal relationships between insulin resistance and endothelial dysfunction may predispose to endothelial dysfunction in the presence of insulin resistance [2].

In our study groups, there was a slight imbalance in sex distribution with a higher female-male ratio in the offspring of normoglycemic subjects compared with the NOPD cohort (2.18 vs 0.82). Because, in general, men tend to have lower vascular reactivity than women, it is possible that these sex differences among the 2 groups may explain some of the differences in vascular reactivity that we observed between NOPD and control groups. However, it seems unlikely that these small differences would significantly alter the main conclusions of our study.

Our findings in the brachial arteries of NOPD are consistent with those of a previous study documenting both endothelial-dependent and -independent abnormalities in the microcirculation of the skin and endothelial-dependent abnormalities in the brachial artery of normoglycemic subjects with diabetic parents [8]. It is important to note that this study also showed that differences in endothelium-dependent dilatation may exist even in absence of differences in insulin sensitivity. By contrast, Balletshofer et al [9] only found significant differences between normoglycemic offspring of diabetic parents and control subjects with respect to FMD and NMD when subgroup analyses were performed to segregate subjects with insulin resistance. Of note, that study

did not report power calculations, and it included only 10 control subjects to compare with 53 subjects with diabetic parents. Because Balletshofer et al did find abnormalities in the subgroup with insulin resistance, a more robust study design with an appropriate number of control subjects may have yielded results similar to our study.

4.3. Circulating markers of inflammation

We found that several circulating markers of inflammation including hsCRP, CD40L, TNF-α, and IL-1β were substantially elevated in the NOPD group compared with the control group. In addition to being markers of inflammation, hsCRP, CD40L, TNF-α, and IL-1β have all been implicated as active mediators of endothelial dysfunction and atherosclerosis [14,27-29]. Moreover, IL-1 and TNF-α have been implicated as mediators of insulin resistance [3,30,31]. Finally, TNF-α inhibits the activity of the adiponectin promoter [32]. As noted earlier, adiponectin levels were lower in NOPD compared with controls. Low plasma adiponectin levels are correlated with insulin resistance [33] and hypoadiponectinemia is correlated with endothelial dysfunction in hypertensive patients [34]. Adiponectin is an adipokine secreted specifically by adipose cells that possess both anti-inflammatory and antiatherogenic properties [35-37] in addition to its metabolic and vascular functions that mimic insulin action [38,39]. When we performed a multivariate analysis of the predictors of FMD in our NOPD group, adiponectin was the only significant predictor of endothelium-dependent vasodilation. Thus, lower adiponectin levels may contribute not only to insulin resistance, but also to a proinflammatory state with endothelial dysfunction, whereas inflammatory mediators such as TNF-α lower expression of adiponectin in a vicious cycle. In the current study, circulating indicators of endothelial cell activation, such as soluble VCAM-1 and ICAM-1, were not different between NOPD and controls. These findings are partially at odds with those previously reported by Caballero et al [8], who observed increased plasma levels of VCAM-1, but not ICAM-1, in NOPD compared with controls. The reasons for this apparent discrepancy are not clear. However, it is possible that our inability to measure VCAM-1 and ICAM-1 in all study subjects (for technical reasons described in "Subjects, materials, and methods") may account, in part, for this difference.

It seems likely that, as with insulin resistance and endothelial dysfunction, there is a genetic component to the elevations in circulating markers of inflammation we observed in the NOPD group. Indeed, it has been estimated that in individuals without acute illness, genetics contributes approximately 40% to the basal levels of hsCRP [17] and that genetic determinants of CRP levels may be independent of traditional risk factors for cardiovascular disease [40]. Of note, circulating hsCRP levels in the NOPD group, although elevated compared with controls, were still within the reference range (<3 mg/L). Thus, the clinical significance of our findings with respect to inflammatory markers is uncertain in the absence of more definitive longitudinal studies.

In summary, young, lean, otherwise healthy offspring of patients with type 2 diabetes mellitus have decreased endothelial-dependent and -independent vascular function, decreased insulin sensitivity, and elevated circulating markers of inflammation compared with matched healthy controls without diabetic parents. More importantly, subjects in the NOPD group were normoglycemic and normotensive without overweight. Thus, our findings are not secondary to frank metabolic and hemodynamic abnormalities, but are likely determined, in part, by genetics underlying interrelated pathophysiologic features of both metabolic and cardiovascular diseases including endothelial dysfunction, insulin resistance, and chronic low-grade inflammatory status. These results may have important implications for identifying populations that can derive substantial benefits from early lifestyle interventions including diet and exercise that are known to improve endothelial function, increase insulin sensitivity, and lower circulating markers of inflammation.

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