Endothelial Dysfunction Is Related to Poor Glycemic Control in Adolescents with Type 1 Diabetes under 5 Years of Disease: Evidence of Metabolic Memory

Gislaine Vissoky Cé, Luis Eduardo Rohde, Antônio Marcos Vargas da Silva, Márcia Khaled Puñales Coutinho, Andrea Cauduro de Castro, and Marcello Casaccia Bertoluci

Programa de Pós-Graduação em Medicina: Ciências Médicas/Universidade Federal do Rio Grande do Sul (G.V.C., M.C.B.), CEP 90035-003, Porto Alegre, Brazil Serviço de Medicina Interna do Hospital de Clínicas de Porto Alegre/Universidade Federal do Rio Grande do Sul (M.C.B.), and Serviço de Cardiologia do Hospital de Clínicas de Porto Alegre/Universidade Federal do Rio Grande do Sul (L.E.R.), CEP 90035-903 Porto Alegre, Brazil; Instituto da Criança com Diabetes/Grupo Hospitalar Conceição (G.V.C., M.K.P.C.), CEP: 91350-250 Porto Alegre, Brazil; Departamento de Fisioterapia e Reabilitação (A.M.V.d.S.), Universidade Federal de Santa Maria, CEP 97105-900 Santa Maria, Brazil; and Laboratório de Bioquímica do Grupo Hospitalar Conceição (A.C.d.C.), CEP: 91350-200 Porto Alegre, Brazil

Context: The relation between endothelial dysfunction (ED), glycemic control, and early type I diabetes mellitus (T1DM) is unclear.

Objective: The objective of the study was to evaluate the association of ED, glycemic control, and the duration of diabetes in T1DM.

Design: This was a cross-sectional study.

Setting: The study was conducted at a public outpatient clinic.

Patients: Fifty-seven T1DM adolescents and 10 healthy age-matched controls participated in the study.

Intervention: There were no interventions.

Methods and Outcome Measures: Endothelial function (ED) was evaluated by flow-mediated dilation (FMD) in the brachial artery after reactive hyperemia. Biochemical data, including HbA1c (glycohemoglobin), high-sensitivity C-reactive protein, lipids, and urinary albumin excretion were collected. Means of four HbA1c values collected at 3-month intervals in the first and second year before FMD analyses were obtained.

Results: Mean FMD was decreased in T1DM compared with controls ($P = 0.023$), independent of age, smoking, hypertension, or dyslipidemia. Twenty-eight of 57 T1DM patients enrolled (49%) had ED. FMD was decreased in microalbuminuric (4.1%) compared with normoalbuminuric patients (10.1%, $P = 0.01$) and controls (14.6%, $P < 0.001$). FMD correlated inversely with mean second-year HbA1c ($r = -0.426$, $P = 0.02$), particularly in patients with less than 5 yr of T1DM ($r = -0.61$, $P = 0.004$). In these patients, high-sensitivity C-reactive protein was strongly correlated with mean first-year HbA1c ($r = -0.66$, $P = 0.0003$). In patients with more than 5 yr of T1DM, we found no significant correlations between ED and glycemic control.

Conclusions: Endothelial dysfunction is common in T1DM adolescents with less than 5 yr of disease. It is associated with duration of disease, microalbuminuria, and mean second-year HbA1c but not with mean first-year HbA1c. These data support the metabolic memory hypothesis. (***J Clin Endocrinol Metab* 96: 1493–1499, 2011**)

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Abbreviations: BMI, Body mass index; ED, endothelial dysfunction; FMD, flow-mediated dilation; HbA1c, glycohemoglobin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; T1DM, type I diabetes mellitus.

Patients with diabetes mellitus are at increased risk for developing microvascular and macrovascular disease (1). Prospective studies indicate that long-term poor glycemic control is an important determinant of these complications because vascular endothelial cells are largely affected by hyperglycemic damage (2–4). Endothelial dysfunction (ED) is an early marker of such injuries and might predict advanced vascular disease (4). In this setting, acute hyperglycemia can directly induce endothelial dysfunction in both normal and diabetic subjects (5, 6). It has been suggested that free radicals such as superoxide generation by the endothelial cells, promoting oxidative stress, may be the chief mechanism involved in acute vascular damage (2). On the other hand, chronic hyperglycemia seems to be a much more complex process, and several pathways are suggested to increase oxidative stress (7).

Recent epidemiological and prospective data support the influence of long-term metabolic control in diabetic micro- and macrovascular complications (2, 8). Data from the Epidemiology of Diabetes Interventions and Complications study (2) showed that patients who were exposed to long-term intensive glycemic control had decreased incidence of vascular complications, even after switching to less intensive treatment. The concept of metabolic memory, implicating that a long-term hyperglycemic environment is memorized for years, was evaluated by Ceriello *et al.* (9), who suggested that glycated mitochondria overproducing free radicals could lead to DNA damage and cell injury and thus alter protein expression turnover in a way that perpetuates the superoxide production cycle.

Systemic inflammation is also a central factor involved in atherosclerosis pathogenesis, the major determinant of macrovascular complications in diabetes (10). In this setting, subclinical inflammation is related to duration and intensity of hyperglycemia. Recent data also support that inflammation can be normalized with insulin therapy in newly diagnosed type 1 diabetes mellitus (T1DM) with short-term hyperglycemia, but like endothelial dysfunction, antioxidant adjunctive therapy to insulin is necessary to normalize inflammatory markers (11).

In the clinical scenario, however, these associations have not been completely unraveled. HbA1c (glycohemoglobin) has been largely used as a marker of chronic hyperglycemia, but in many studies there appears to be only a weak association to ED and subclinical inflammation. An inverse correlation has been observed between HbA1c and flow-mediated dilation (12), but an unexpected positive correlation has also been described (13). This discrepancy may be attributed in part to the fact that HbA1c evaluates a relatively short period of glycemic control, whereas ED might be the result of a longer cell injury process. Few studies have directly compared the effect of short and long periods of hyperglycemia on endothelial dysfunction and vascular inflammation specifically in adolescents with recent T1DM.

Thus, the main objective of the present study was to evaluate the association of ED with short- and long-term glycemic control in adolescents with T1DM of less than or greater than 5 yr of disease. Subclinical systemic inflammation and microalbuminuria were also evaluated.

Materials and Methods

Study design and patients

We performed a cross-sectional study involving 57 patients with T1DM and disease duration of less than 10 yr. Ten nondiabetic, age-matched adolescents were also enrolled as controls. Patients were consecutively recruited from the outpatient clinic at a public health service for the treatment of diabetes in infancy and adolescence. Data were collected from October 2007 to November 2008. From the T1DM patients who were screened, 60 who fulfilled inclusion criteria were invited to participate in the study, but three declined written consent or assent and 57 patients were enrolled.

T1DM was clinically defined when diabetes ketoacidosis was diagnosed before 20 yr of age, body mass index (BMI) was less than 28 kg/m^2, in the absence of a family history of diabetes mellitus and whether insulin therapy was required in the first year of diagnosis. Inclusion criteria were BMI from 16 to 28 kg/m^2, a late pubertal Tanner stage (IV or V), current intensive insulin therapy, high motivation, and the ability to perform intensive blood glucose self-monitoring. T1DM patients were then classified as having diabetes for less than 5 yr or greater than 5 yr for further statistical analyses. Exclusion criteria were current or past tobacco use; clinical hypertension; known hypothyroidism; current oral medication, including any kind of hormonal contraception; current or past pregnancy; and any recent or past history of inflammatory disease, including known clinical inflammatory bowel disease such as Crohn's disease, rheumatoid arthritis, chronic thyroiditis, asthma, allergies, cancer, or clinically significant vascular disease. The study was approved by the local ethics committee. All adolescents signed an informed assent, and their respective guardians filled out and signed a written informed consent.

Microalbuminuria was defined as urinary albumin concentration 30 mg/g of creatinine or greater or urinary albumin excretion 30 mg per 24 h or greater, in three separate first-morning samples in the absence of urinary tract infection, according to the guidelines of the American Diabetes Association (14). All patients were evaluated by an experienced ophthalmologist for the presence of retinopathy.

Research protocol

In the first screening visit, patients were informed about the study, and assent and consent forms were filled out and signed by both patients and guardians, respectively. In a second visit, a complete clinical interview and physical examination were performed. Anthropometric data including BMI and waist circumference were determined. The patients were asked to remain on

their current insulin regimen and perform intensive self-monitoring blood glucose for 30 d before endothelial function determination as follows: [premeal (daily); 2 h postprandial at breakfast, lunch, and dinner (daily); and at 0300 h (weekly)]. Blood pressure was performed using a mercury sphygmomanometer by a single examiner (G.V.C.). Measurements were made while in the sitting position, using the mean of three recorded values.

Endothelial function evaluation

Endothelial function was evaluated 30 d after the first visit. Blood was drawn for biochemical determinations after a 12-h fasting period, and their usual insulin dosage was administered. Examinations were performed in the morning, after a light breakfast, consisting of 200 ml skim milk, 1 slice (15 g) of wholegrain bread, and 10 g of cottage cheese. No caffeine or high-fat foods were allowed. Endothelial function was determined by the measurement of flow-mediated dilation (FMD) in the brachial artery, using a high-resolution vascular ultrasound (EnVisor CHD; Philips, Bothell, WA) and a 3- to 12-MHz linear-array transducer (L12–3; Philips), according to internationally accepted guidelines (15). This technique was previously described by our group elsewhere (16), and the validity of this method was confirmed in previous studies (11, 17, 18). All measurements were performed by the same person (A.M.V.d.S.). Briefly, the patient rested comfortably in a supine position in a room with controlled temperature (at 24 C) with the left arm relaxed and extended laterally on a soft base. Systemic blood pressure was measured by a mercury sphygmomanometer in the contralateral arm, by the same person (G.V.C.). A baseline scan was performed and the anterolateral diameters of the brachial artery were measured with the transducer placed 5 cm above the antecubital fossa. Reactive hyperemia (endothelium dependent dilation) was induced by inflating the cuff 50 mm Hg above the systolic blood pressure for 5 min. The brachial artery was scanned in the same position 45–60 sec after the cuff was deflated and the artery diameters were again measured. Measurements were taken in triplicate at end diastole in three consecutive cycles, coinciding with the electrocardiogram R-wave. Maximal percent dilation from baseline [peak FMD (percent)] was then assessed. ED was considered to be present if peak FMD was 8% or less from baseline (19). After 30 min of rest, new baseline measurements were repeated, a sublingual nitrate (0.3 mg) spray was administered, and the maximal non-endothelial-dependent dilation was assessed 4 min later. Smooth muscle dysfunction was considered when nitrate mediated dilation was 8% or less (19). Images were analyzed by the same investigator who was blinded to clinical data (A.M.V.d.S.). Intraobserver variability was 1.7%.

Biochemical measurements

Urinary albumin excretion and HbA1c were determined by immune turbidimetry (National Glycohemoglobin Standardization Program certified autoanalyzer-Cobas Integra 400; Roche, Stockholm, Sweden). Plasma glucose was analyzed by the glucose-peroxidase method through colorimetric enzymatic reactions. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride concentrations were also measured by the colorimetric enzymatic method (Modular; Roche, Mannheim, Germany). Creatinine was measured by the Jaffe method (Modular; Roche) and high-sensitivity C-reactive protein (hs-CRP) by nephelometry (BN II; Dade-Behring, Deerfield, IL).

HbA1c historical data

Historical data from HbA1c at 3, 6, 9, 12, 15, 18, and 24 months before endothelial evaluation were obtained from routine medical records. All HbA1c values were measured at the same institution, using the same National Glycohemoglobin Standardization Program-certified analytical technique. Quality control of HbA1c measurement throughout the 24 months was

TABLE 1. Clinical and biochemical characteristics of type 1 diabetes patients and controls

		Type 1 Diabetes				
	Controls	ED	Without ED	All	*P* value[a]	*P* value†
N	10	28	29	57		
Age (yr)	20.2 ± 3.7	17.4 ± 3.4	18.0 ± 3.9	17.7 ± 3.7	0.54	0.051
Male/female (%)	50/50	39/61	51/49	45/55	0.33	0.87
Duration of diabetes (months)	NA	105.4 ± 74.7[a]	66.3 ± 48.0	85.1 ± 65.0	0.02	NA
BMI (kg/m^2)	21.6 ± 3.1	22.4 ± 2.53	22.4 ± 2.57	22.3 ± 2.5	0.99	0.418
Abdominal waist (cm)	73 ± 8	78 ± 7.5	77 ± 6.3	78 ± 6.8	0.50	0.064
SBP (mm Hg)	112 ± 9	106 ± 12	106 ± 10.5	106 ± 11	0.94	0.12
HbA1c (%)	5.13 ± 0.22	8.97 ± 1.85[b]	8.23 ± 1.45[b]	8.59 ± 1.66[b]	0.10	<0.001
Fasting blood glucose (mg/dl)	84.8 ± 11	192 ± 84[b]	187 ± 87.5[b]	189 ± 83[b]	0.80	<0.001
hs-CRP (mg/liter)	2.49 ± 3.0	2.16 ± 3.0	1.74 ± 1.9	1.95 ± 2.5	0.52	0.53
Total cholesterol (mg/dl)	169 ± 43	167 ± 41	159 ± 28	163 ± 35	0.37	0.64
LDL cholesterol (mg/dl)	94 ± 41	95 ± 32	91 ± 26	93 ± 29	0.56	0.95
HDLc cholesterol (mg/dl)	54 ± 25	57 ± 13	56 ± 15	56 ± 14	0.79	0.69
Triglycerides (mg/dl)	118 ± 47	84 ± 59[b]	72 ± 30[b]	78 ± 47[b]	0.34	<0.001
Serum creatinine (mg/dl)	0.78 ± 0.16	0.76 ± 0.14	0.79 ± 0.19	0.77 ± 0.17	0.52	0.85
Urinary albumin/creatinine (mg/g)	5.8 ± 7.8	53 ± 15.3	26 ± 5.3	40.3 ± 116.4	0.79	0.08
Microalbuminuria (%)	NA	22.2[a]	3.5	12.3	0.04	NA
Retinopathy number (%)	NA	1 (3.6)	2 (6.9)	3 (5.2)	0.98	NA

Data are mean ± SD. SBP, Systolic blood pressure; NA, nonapplicable.

[a] ED *vs.* without ED.

[b] T1DM *vs.* controls.

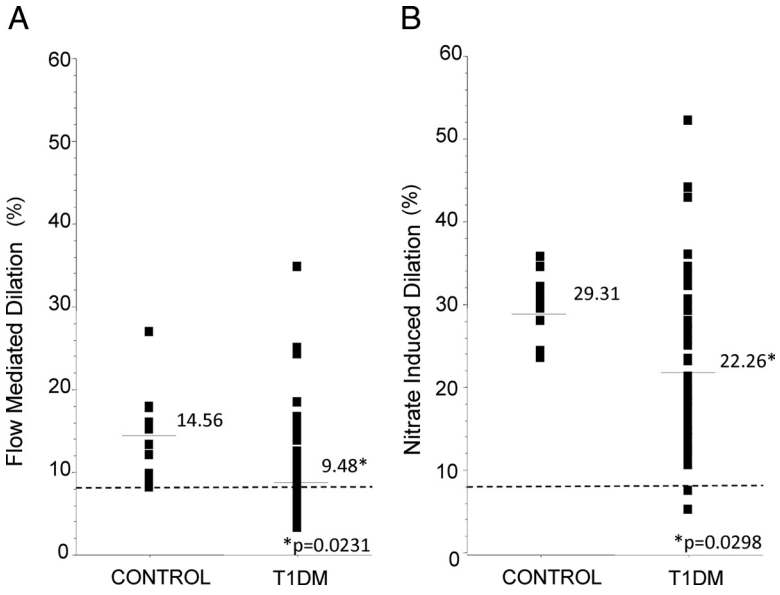

FIG. 1. FMD (endothelial dependent) (A) and nitrate-induced dilation (nonendothelial dependent) (B) in patients with T1DM (n = 57) and nondiabetic controls (n = 10). *Black lines* and *numbers* depict the mean. *Horizontal dashed lines* represent normal values.

carefully monitored by a blinded member of the medical team (A.C.d.C.), and all enrolled patients had complete HBA1c data.

Statistical analysis

Data were expressed as mean and SD or absolute numbers and percentages. Variables with normal distribution were compared by the unpaired Student's *t* test, and categorical variables were compared by χ^2 statistic. The nonparametric Mann-Whitney test was used to analyze urinary albumin-to-creatinine ratio (milligrams per gram) and hs-CRP. Comparisons of data among groups were performed using one-way ANOVA followed by a Bonferroni test and multiple comparison tests. For correlation analysis, Pearson's correlation coefficients (r) were calculated. For statistical purposes, the mean of four independent HbA1c samples obtained at 0, 3, 6, and 9 months before endothelial function evaluation was considered mean first-year HbA1c, whereas the mean of four HbA1c samples collected at 12, 15, 18, and 24 months before the vascular test was defined as mean second-year HbA1c. A $P < 0.05$ was considered statistically significant.

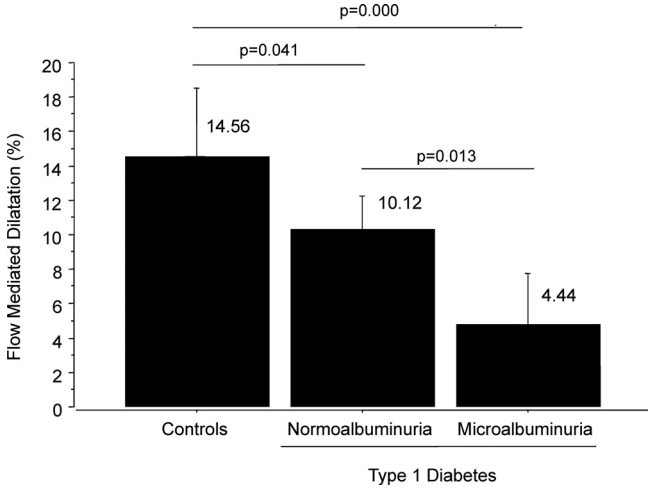

FIG. 2. FMD in controls, normoalbuminuric, and microalbuminuric T1DM patients. *Black bars* represent the mean and *lines* the SD.

Results

T1DM *vs.* controls

Clinical and biochemical characteristics of T1DM patients and controls were compared in Table 1. No significant differences were found between T1DM patients and controls with respect to age, BMI, abdominal waist circumference, systolic and diastolic blood pressure, hs-CRP, serum creatinine, serum total cholesterol, LDL cholesterol, and HDL cholesterol levels. Mean triglyceride level was decreased in T1DM patients. Mean FMD was significantly decreased in T1DM patients compared with nondiabetic controls (9.5 ± 6.5 *vs.* 14.6 ± 5.6%, respectively, $P = 0.023$) (Fig. 1A). Mean nitrate induced dilation was also significantly decreased in T1DM patients compared with controls (22.3 ± 9.8 *vs.* 29.3 ± 4.2%, $P = 0.02$). Two T1DM patients had true smooth muscle dysfunction (Fig. 1B).

T1DM with ED *vs.* without ED

Twenty-eight of the 57 T1DM patients enrolled (49%) had ED, defined as peak FMD 8% or less (Table 1). No significant differences between T1DM patients with or without ED were observed for most clinical characteristics, such as age, BMI, abdominal waist circumference, systolic and diastolic blood pressure, fasting blood glucose, lipid profile, hs-CRP, or the presence of retinopathy. Microalbuminuria was more prevalent in patients with ED than in those without ED (Table 1). FMD was decreased in microalbuminuric compared with normoalbuminuric patients ($P = 0.013$) and controls ($P < 0.001$): 4.4 ± 4.1 *vs.* 10.1 ± 6.5% *vs.* 14.6 ± 5.6%, respectively (Fig. 2). The duration of diabetes was also significantly longer in patients with ED when compared with patients without ED (Table 1). A significant inverse linear correlation between duration of diabetes and FMD was found (r = −0.293, $P = 0.0264$).

Glycemic control *vs.* ED

Considering the whole group of T1DM patients, HbA1c at the time of endothelial function analysis was similar between those with and without ED (8.2 ± 0.9 *vs.* 8.0 ± 1.4%, respectively; $P = 0.66$), whereas mean second-year HbA1c was significantly higher in ED compared with non-ED (9.6 ± 2.4 *vs.* 8.1 ± 1.3%, respectively; $P = 0.048$). Moreover, FMD was inversely correlated with

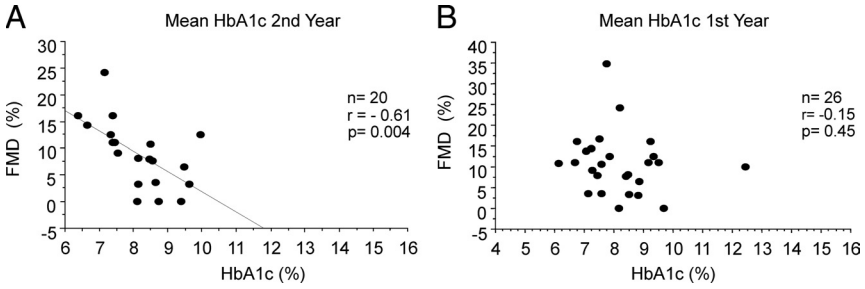

FIG. 3. Scatter plots between FMD and mean second-year HbA1c (A) and mean first-year HbA1c (B) in patients with less than 5 yr of T1DM.

mean second-year HbA1c ($r = -0.287$, $P = 0.031$) but not with mean first-year HbA1c ($r = -0.126$, $P = 0.37$).

Duration of diabetes and ED

In patients with less than 5 yr of T1DM, ED was a common finding (35.7%), but it was more prevalent in patients with longer duration of T1DM (60%, $P < 0.01$). At the moment of FMD determination, patients with less than 5 yr of diabetes had lower HbA1c (8.06 ± 1.22 *vs.* 9.10 ± 1.92%; $P = 0.02$), lower hs-CRP (1.25 ± 0.88 *vs.* 2.62 ± 3.23 mg/liter; $P = 0.03$), and smaller abdominal waist circumference (75.7 ± 6.7 *vs.* 79.6 ± 6.6 cm; $P = 0.03$) than patients with more than 5 yr of T1DM duration. There were no other significant differences in clinical or biochemical characteristics between patients with and without ED in this group.

In patients with less than 5 yr of T1DM, mean second-year HbA1c was higher in those with ED compared with those without ED (8.8 ± 0.6 *vs.* 7. 6 ± 1.0%, respectively; $P = 0.005$), whereas mean first-year HbA1c was not different (8.3 ± 0.8 *vs.* 8.1 ± 1.5%, respectively; $P = 0.66$). In fact, mean second-year HbA1c correlated inversely with FMD ($r = -0.61$, $P = 0.004$), whereas mean first-year HbA1c did not ($r = -0.15$, $P = 0.45$) (Fig. 3, A and B, respectively). Figure 4 depicts the behavior of FMD in T1DM patients whether in good, intermediate, or poor glycemic control according to mean first- and second-year HbA1c. We observed a stepwise decrease in FMD according to categories of mean second-year HbA1c control in patients with T1DM with less than 5 yr of disease (Fig. 4A). No such difference in FMD was observed in respect to mean first-year HbA1c (Fig. 4B). In patients with more than 5 yr of T1DM, no significant correlations were found between ED, either with mean first-year HbA1c ($r < 0.1$, $P = 0.98$) or second-year HbA1c ($r < 0.1$, $P = 0.71$).

Inflammation and ED

hs-CRP was not different in comparisons between all T1DM patients and controls or between T1DM patients with or without ED (Table 1). However, hs-CRP was higher in patients with more than 5 yr of T1DM than in patients with less than 5 yr (2.6 ± 3.2 *vs.* 1.25 ± 0.9 mg/liter; $P = 0.03$) (data not shown). In stratified analysis by diabetes duration, hs-CRP correlated marginally with mean second-year HbA1c in those with less than 5 yr of diabetes (Fig. 5A) but was strongly correlated with mean first-year HbA1c ($r = -0.66$, $P = 0.0003$, Fig. 5B). There was no correlation between mean first- or second-year HbA1c and hs-CRP in patients with more than 5 yr of diabetes.

Discussion

In this study, we observed that endothelial dysfunction in T1DM is an early phenomenon that is relatively common in adolescents with recent onset of diabetes, independent of age, smoking, hypertension, or hyperlipidemia. This finding was particularly influenced by glycemic control and duration of the disease. Our data suggest that intermediate-term rather than short-term glycemic control has a major influence on ED in the early years of T1DM, represented in our study by mean second- and first-year HbA1c, respectively. Systemic subclinical inflammation, assessed by hs-CRP, on the other hand, was more related to short-term glycemic control.

The mechanism by which chronic hyperglycemia is associated with ED is complex and not completely understood. Oxidative stress, polyol pathway activation, protein kinase C system activation, and the presence of ad-

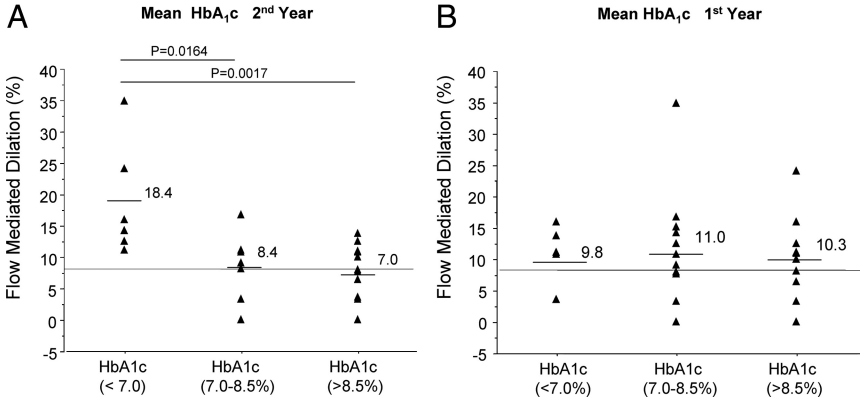

FIG. 4. FMD in patients with good (HbA1c < 7%), intermediate (HbA1c 7–8.5%), and poor (HbA1c > 8.5%) glycemic control according to mean second-year HbA1c (A) and mean first-year HbA1c (B) in patients with less than 5 yr of T1DM. *Dashed bars* represent normal values.

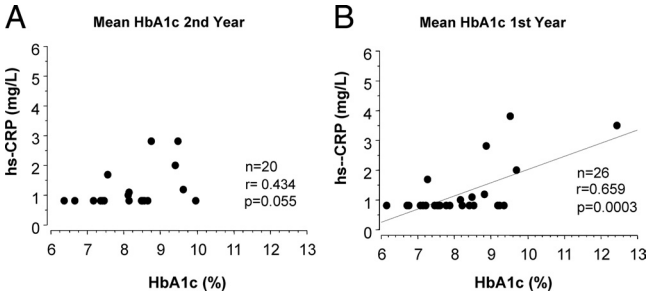

FIG. 5. Scatter plot showing the relationship of hs-CRP and mean second-year HbA1c (A) and mean first-year HbA1c (B) in patients with less than 5 yr of T1DM.

vanced glycation end-products are all potential mechanisms involved (4, 20, 21). The concept of metabolic memory was recently proposed by Ceriello *et al.* (9), who pointed out that mechanisms propagating this phenomenon appear to be related to the nonenzymatic glycation process and to the excess of cellular reactive oxygen and nitrogen species, originating at the level of glycated mitochondrial proteins, and acting synergistically to maintain stress signaling independent of glucose levels. Our findings are in accordance with the concept of metabolic memory. Intermediate and long-term sustained poor glycemic control seems to be a more important determinant of ED than short-term glycemic control. Considering that slow pathways such as advanced glycosylation end-product formation may need a longer time to develop, it seems reasonable to speculate that the temporal range evaluated by HbA1c (~3 months) might not be enough to have a major impact on all metabolic processes that lead to ED. This may explain, at least in part, the discrepancies observed in the literature among studies correlating HbA1c and endothelial function.

Previous studies have suggested that the disease duration and microalbuminuria may be related to ED (22). Although ED is common in patients with T1DM after 10 yr of disease (17, 23), its presence before 5 yr of diabetes has been challenged. Two studies indicated that it may occur in the first years after the diagnosis (17, 24), but another report did not corroborate this observation (13). Järvisalo *et al.* (24) observed that 36% of children with T1DM and poor metabolic control (mean HbA1c of 8.9%) had ED before 4 yr of disease (25). In addition, Dogra *et al.* (17) examined whether endothelial function was impaired in T1DM under conditions of near-normoglycemia, comparing FMD with age-matched healthy control subjects. These investigators observed that FMD was significantly lower in patients with microalbuminuria compared with those with normoalbuminuria and controls. In contrast, Ladeia *et al.* (13) compared adolescents with up to 3 yr of T1DM (mean HbA1c 9.35%) with nondiabetic individuals and found no differences in FMD. Our results concur with data from Järvisalo *et al.* demonstrating a remarkably similar prevalence of ED in adolescents up to 5 yr of disease (36%). Our data also indicate that ED is significantly associated with microalbuminuria, suggesting that it is a phenomenon that occurs quite early in the natural history of T1DM microvascular complications, supporting the hypothesis that it may even be a precursor of microvascular disease.

A potential limitation of the study is that the enrollment of patients in the study could theoretically promote better glycemic control in the 30-d run-in phase due to an intensification of self-monitoring blood glucose, thus tending to minimize differences in short-term control among diabetic subjects in the recent glycemic control period. We aimed to minimize this potential bias by using the mean of four measurements of HbA1c in the first-year and comparing it with the mean of four HbA1c in the second year. This may have decreased the impact of recent improvement of glycemic control in FMD. Moreover, when we analyzed correlations between FMD and HbA1c at 3, 6, 9, and 12 months individually, no correlation with FMD appeared before 15, 18, and 24 months, indicating that recent amelioration (in the last 30 d) had minimal impact on FMD.

In conclusion, endothelial dysfunction is an early phenomenon in T1DM patients. It is associated with duration of disease and microalbuminuria and is more closely related to mean HbA1c in the previous 2 yr than with mean HbA1c in previous year, an effect that may be related to the so-called metabolic memory. Subclinical chronic inflammation, on the other hand, is more affected by mean HbA1c in the previous year. Glycemic control, subclinical chronic inflammation, and endothelial function are all players in a complex interplay that evolves over time, setting the stage for the development of micro- and macrovascular complications. Our findings emphasize that intensive glucose control in the early years of diabetes may be essential to prevent future vascular complications in T1DM.

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Address all correspondence and requests for reprints to: Marcello Casaccia Bertoluci, M.D., Ph.D., Hospital de Clínicas de Porto Alegre, Serviço de Medicina Interna, Rua Ramiro Barcelos 2350, Sala 700, 7° andar Porto Alegre RS, CEP 90035-903 Brazil. E-mail: mbertoluci@uol.com.br.

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