Longitudinal evaluation of endothelial function in children and adolescents with type 1 diabetes mellitus: A long-term follow-up study

Patrizia Bruzzi,1 Barbara Predieri,1 Viviana Dora Patianna,1 Annamaria Salvini,1 Rosario Rossi,2 Maria Grazia Modena2 and Lorenzo Iughetti1

Departments of 1Pediatrics and 2Cardiology, University of Modena and Reggio Emilia, Modena, Italy

Abstract

Background: Type 1 diabetes (T1DM) predisposes to cardiovascular disease, increasing the risk to develop atherosclerosis. In the pediatric population, the cardiovascular risk may be evaluated examining endothelial function by a non-invasive ultrasound technique, namely flow-mediated dilation (FMD) of the brachial artery. The aims of this study were the longitudinal evaluation of the potential change in the endothelium-dependent vasomotor function in children and adolescents with T1DM and the identification of clinical and laboratory data correlated to modifications.

Methods: We studied 39 T1DM patients (20 girls and 19 boys; aged 11.2 ± 3.72 years). FMD and blood samples were obtained from all patients at baseline (time 0) and after a follow up of at least 1 year (time 1). FMD was also evaluated in 45 healthy controls (22 boys, 23 girls) aged 10.2 ± 3.05 years.

Results: At time 0, 43.6% of T1DM patients presented an impaired FMD. FMD at time 1 revealed a dramatic impairment of endothelial function: altered FMD values were shown in 61.5% of patients and it got worse in 74.3% of them. Longitudinally, boys had a greater impairment of FMD than girls. At baseline, multivariate analysis identified only sex as significant predictor of FMD ($\beta = 0.470, P = 0.029$).

Conclusions: Because endothelial dysfunction appears earlier in diabetic children, they are at higher risk to develop atherosclerosis. Our results suggest the usefulness of FMD as a tool to stratify pediatric T1DM patients according to their cardiovascular risk and to follow them up longitudinally.

Key words atherosclerosis, endothelial function, flow-mediated dilation, glycosylated hemoglobin, type 1 diabetes mellitus.
disease with current coronary aneurysms.\textsuperscript{11} The Diabetes Control and Complications Trial (DCCT) and its follow up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, showed that the risk of micro- and macro-vascular complications linked to T1DM is related to long-term glycemic control.\textsuperscript{12–14} Although achievement of optimal glycemic control has both immediate and long-term health advantages,\textsuperscript{12–14} glycemic control remains suboptimal in a large subset of the population of individuals with T1DM. For example, children and adolescents with T1DM consistently demonstrate clinic-wide HbA\textsubscript{1c} values higher than adults.\textsuperscript{18} It demonstrates that, even on intensive insulin treatment, the majority of adolescents in DCCT demonstrate improvements in glycemic control as a result of the intensive insulin regimen; however, both the intensively treated adolescents and those who received standard care have mean HbA\textsubscript{1c} values higher than adults.\textsuperscript{18} It demonstrates that, even on intensive insulin treatment, the majority of children and adolescents with T1DM are unable to maintain a near-normal glycemic control.\textsuperscript{18} Because vascular complications of diabetes are not yet clinically evident in diabetic children, surrogate markers for CHD are needed to check the efficacy of treatment.\textsuperscript{19}

The aims of our study were the evaluation of the potential changes in the endothelium-dependent vasomotor function in children and adolescents with T1DM and the identification of clinical and laboratory correlated and predictive factors.

\section*{Methods}

\textbf{Patients}

We studied 39 children and adolescents with T1DM (20 girls and 19 boys, aged 11.2 ± 3.72 years [range: 4.92–18.5]), with T1DM duration of 48.05 ± 33.13 months (range 4–114) recruited from T1DM patients followed up at our Pediatric Diabetes Clinic, and 45 age-matched healthy control children (23 girls and 22 boys, aged 10.2 ± 3.05 years [range: 6–16]).

Controls were selected from children and adolescents who visited our clinic for a suspected endocrinological, auxological or metabolic problem, which was not confirmed.

In all included subjects, height, weight, pubertal staging, laboratory and ultrasonographic study were evaluated at baseline (Time 0) and in only T1DM patients also after a period >1 year (Time 1).

The clinical characteristics of the patients are reported in Table 1. All subjects were normal weight and none had other acute or chronic diseases. Thyroid hormones, hepatic and renal functions were normal. All diabetic patients were taking no medications other than insulin; none had retinopathy, demonstrated by fundoscopic examination, nor microalbuminuria, measured by overnight albumin excretion rate. Subjects with hypertension or history of smoking were excluded from the study.

\textbf{Methods}

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Harpenden, Crummych, UK). Bodyweight was measured to the nearest 0.1 kg and body mass index (BMI) was obtained from the weight in kg/height in meters squared and expressed as Z-score with respect to chronological age (CA).

Pubertal development was determined using the grading system defined by Tanner for pubic hair (PH) and breast (B).\textsuperscript{20}

After an overnight fast, each subject underwent blood sample collection in the morning before insulin administration and the ultrasound test. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) plasma concentrations were measured by standard enzymatic methods.

Fasting plasma glucose level was measured by the hexokinase spectrophotometry method (Synchron System, Beckman Instruments, Brea, CA, USA) and HbA\textsubscript{1c} was determined as the average value of the last four determinations performed by high-performance liquid chromatography in the last year (Variant Analyser, Bio-Rad, DCA Analyzer, Siemens AG, Erlangen, Germany). HbA\textsubscript{1c} values were standardized according to DCCT.\textsuperscript{21}

All children and adolescents underwent an ultrasonography in order to evaluate endothelial-dependent vasodilation, using the determination of the FMD of the brachial artery. To reduce the inter-observer intersession coefficient of variation, one single, experienced vascular ultrasonographer performed all exams. The study of the brachial artery was performed in all participants by means of an Acuson 128 XP/10 mainframe (Acuson, Mountain View, CA, USA) with a 7.0-MHz linear array transducer. The technique for assessing FMD has been described in detail elsewhere.\textsuperscript{6,18,22,23} Briefly, FMD was assessed in the subject’s right arm in the recumbent position after a 15-min equilibration period in a temperature-controlled room (22–25°C). The artery was longitudinally imaged approximately 5 cm proximal to the antecubital crease, and brachial artery diameter (BAD) was measured at end-diastole. A suitable site for imaging the vessel was first selected, with reproducible ultrasonic markers, such as venous valves or vessel bifurcations, to ensure that measurement occurred at the same place for each scan. An electrocardiogram was recorded with the ultrasonic images. After the baseline resting scan, a pneumatic cuff placed at the level of the mid-forearm (proximal to the target artery) was inflated until no blood flow was detected through the brachial artery with the Doppler probe, and this pressure was held for 5 min. Increased flow was then induced with sudden cuff deflation and a continuous scan was performed for 1 min. Arterial flow velocity was measured by means of a pulsed Doppler signal at 60° to the vessel, during the

\begin{table}[h]
\centering
\caption{Baseline clinical characteristics of patients with T1DM compared to healthy controls}
\begin{tabular}{lccc}
\hline
Parameter & T1DM group & Control group & \(P\) \\
\hline
Age (years) & 11.2 ± 3.72 & 10.2 ± 3.05 & 0.202 \\
Height (cm) & 146.7 ± 21.3 & 143.2 ± 13.1 & 0.460 \\
Weight (kg) & 43.4 ± 15.8 & 38.8 ± 12.3 & 0.213 \\
Body mass index (kg/m\(^2\)) & 19.3 ± 2.53 & 18.4 ± 2.76 & 0.186 \\
SBP (mmHg) & 106.4 ± 12.6 & 100.1 ± 14.5 & 0.071 \\
DBP (mmHg) & 68.9 ± 9.59 & 64.2 ± 11.1 & 0.081 \\
\hline
\end{tabular}
\end{table}
resting scan, and for the first 15 s after deflation of the cuff. For the reactive hyperemia scan, BAD measurements were taken 45–60 s after cuff deflation. For each scan, measurements were made over four cardiac cycles, and the measurements were averaged. FMD was calculated from the diameters as (reactive hyperemia – baseline)/baseline percent.

**Statistics**

All results are reported as the mean ± standard deviation (SD). Statistical analysis (Statistica, StatSoft, Tulsa, OK, USA) was performed using the Student’s t-test for paired and unpaired samples, when appropriate, to evaluate statistical differences between basal and follow-up data and between analyzed groups, respectively. Pearson’s correlation and logistic regression were also performed to analyze the correlation among variables. A P-value below 0.05 was considered statistically significant.

The association between potential predictors and FMD values at the time of evaluation was analyzed considering the following two multivariate logistic regression models:

1. **FMD Time 0**
   a. sex, chronological age, months of disease, height standard deviation score (SDS), BMI SDS, pubertal status (yes/no), BAD.
   b. TC, HDL-C, LDL-C, TG, glycemia, mean HbA1c, beginning HbA1c.

2. **FMD Time 1**
   a. sex, chronological age, months of disease, height SDS, BMI SDS, pubertal status (yes/no), BAD.
   b. TC, HDL-C, LDL-C, TG, glycemia, mean HbA1c, beginning HbA1c.

**Results**

**Clinical data**

Table 1 shows the clinical characteristics of patients with T1DM compared to healthy controls at baseline. No significant differences were found.

**Biochemical data and endothelial function evaluation**

At the first evaluation (time 0) diabetic patients showed an HbA1c value of 8.01 ± 0.92%. No alteration of lipids levels was detected. After 37.02 ± 14.9 months of follow up (time 1), the metabolic control of T1DM did not change significantly (Table 2).

Evaluation of FMD at baseline was within the normal range (more than 7%) in 56.4% of T1DM patients. No significant difference was detected in FMD values between T1DM and control groups (8.45 ± 8.89 vs 8.95 ± 7.74%, respectively; P = 0.793). Through follow up, 74.3% T1DM patients worsened their endothelial function, achieving the 61.5% of population with an impaired (less than 7%) FMD (Fig. 1). Figure 2 shows the significant impairment of FMD during the follow-up period in the T1DM group (8.45 ± 8.89 vs 3.92 ± 8.61%, respectively; P = 0.011) concomitant to a significant enlargement of BAD (2.80 ± 0.63 vs 3.03 ± 0.76 mm, respectively; P < 0.001) and no other metabolic and biochemical change (Table 2).

---

**Table 2** Comparison between diabetic patients at baseline and after follow up

| Parameter | Time 0 (baseline) | Time 1 (after a follow up of 37.02 ± 14.9 months) | P |
|-----------|------------------|-----------------------------------------------|---|
| Auxological data | | | |
| Height SDS | 0.54 ± 1.21 | 0.37 ± 1.30 | 0.080 |
| BMI SDS | 0.66 ± 0.66 | 0.66 ± 0.76 | 0.977 |
| Pre-pubertal stage (% patients) | 35 | 10 | - |
| Glycemic control | | | |
| Fasting plasma glucose (mg/dl) | 245.5 ± 94.8 | 210.7 ± 95.0 | 0.084 |
| Mean HbA1c (%) | 8.01 ± 0.92 | 8.08 ± 1.20 | 0.561 |
| Duration of diabetes (months) | 44.9 ± 32.1 | 82.0 ± 33.2 | <0.001 |
| Lipids profile | | | |
| Total cholesterol (mg/dl) | 162.3 ± 36.1 | 160.5 ± 31.8 | 0.606 |
| HDL cholesterol (mg/dl) | 63.2 ± 11.9 | 61.7 ± 12.7 | 0.442 |
| LDL cholesterol (mg/dl) | 87.1 ± 33.1 | 82.8 ± 31.0 | 0.284 |
| Triglycerides (mg/dl) | 58.4 ± 23.3 | 67.0 ± 28.2 | 0.065 |
| Endothelial function | | | |
| BAD (mm) | 2.80 ± 0.63 | 3.03 ± 0.76 | <0.001 |
| FMD (%) | 8.45 ± 8.89 | 3.92 ± 8.61 | 0.011 |

Data are mean value ± one SD or %. BAD, baseline artery diameter; BMI, body mass index; FMD, flow-mediated dilation; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SDS, standard deviation score.

Table 3 summarizes FMD changes between sexes among T1DM patients. At baseline, FMD mean values were comparable in boys and girls, but during follow up, FMD dramatically decreased only in boys. The statistical significance of FMD worsening persists within the single group of boys over time as well as compared with the female group at time 1 (Table 3). In both sexes, BAD increased significantly during follow-up in respect to
basal values. Interestingly, at the end of follow up, girls presented an increased BMI SDS (0.90 ± 0.62 vs 0.41 ± 0.82 SDS, respectively; \( P = 0.044 \)) and worse TC levels (171 ± 29.46 vs 149.47 ± 31.07, respectively; \( P = 0.032 \)) than boys did. Nevertheless, it is important to notice that all these values were within the normal range for sex and age in all the study population. Glycemic control (HbA1c at T1DM diagnosis, mean glycemic values and HbA1c mean values), chronological age and duration of T1DM did not differ between sexes during the study period (data not shown).

In these findings, using Spearman correlation in T1DM patients, no correlation was detected between FMD and any clinical and metabolic variable, apart from chronological age at time 0 (\( r = 0.32, P = 0.048 \)) and HbA1c when T1DM has been diagnosed at time 1 (\( r = -0.38, P = 0.01 \)) (Fig. 3). No correlation was documented between FMD and BAD at the beginning of our study and at follow up. This suggests that the previously described differences in BAD did not significantly influence the concomitant changes in FMD.

According to pubertal status, 14 of 39 and four of 39 patients were pre-pubertal at time 0 and time 1, respectively. There were no significant differences in metabolic parameters and in endothelial function between these subgroups (data not shown).

Table 4 summarizes the results of the multiple regression analyses performed in T1DM patients. As data at time 1 show, only sex, and specifically male sex, has to be considered a significant negative predictor for FMD impairment over time (\( P = 0.029, \beta = 0.470 \)).

Discussion

The present study demonstrates that an impaired endothelial function is common in T1DM children and adolescents, even a few years after the onset of diabetes. Moreover, our longitudinal results underline that FMD rapidly impaired despite a glycemic control comparable with the control of patients in the active arm of DCCT.

A cardiovascular event is rare in early childhood, but risk factors are present in pediatric age, predicting CHD in adulthood.24-26 The impairment of endothelial function in patients with T1DM was demonstrated first in adults and subsequently confirmed in adolescents. Even though recent advances in diabetes treatment have been successful in decreasing morbidity and mortality from diabetes-related retinopathy, nephropathy and neuropathy,27 there are clear evidences for the lack of improvement in mortality for CHD.28 Consequently, a valid marker identifying patients with a high CHD risk seems to be a fundamental tool in the future management of T1DM pediatric patients. Donaghue et al.29 suggested, for example, that in adolescents with T1DM, a large vessel diameter was the most common damage. Meeking et al.30 and Hooper et al.31 clearly showed that...
Table 4. Multiple regression analyses performed at baseline (time 0) and at follow-up (time 1) in the T1DM study population.

| Model 1 – Regression coefficient | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se
|----------------------------------|--------|----|--------|----|--------|----|--------|----|--------|----|--------|----|--------|----|--------|----|--------|----|
| FMD Time 0                        | 2.11   | 3.50 | 1.18   | 0.69 | 0.004  | 0.04 | 0.004  | 0.04 | 0.01   | 0.01 | 0.01   | 0.01 | 0.01   | 0.01 | 0.004  | 0.004 | 0.004  | 0.004 |
| FMD Time 1                        | −10.2  | 3.27 | 0.02   | 0.03 | 0.003  | 0.003| 0.003  | 0.003| 0.003  | 0.003| 0.003  | 0.003| 0.003  | 0.003| 0.003  | 0.003| 0.003  | 0.003|
| Model 2 – Regression coefficient | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se | Coeff. | se
| FMD Time 0                        | −0.47  | 0.82 | 0.32   | 0.32 | 0.37   | 0.37 | 0.19   | 0.19 | 0.15   | 0.15 | 0.10   | 0.10 | 0.01   | 0.01 | 0.01   | 0.01 | 0.01   | 0.01 |
| FMD Time 1                        | 0.01   | 0.10 | 0.001  | 0.001| 0.01   | 0.01 | 0.001  | 0.001| 0.001  | 0.001| 0.001  | 0.001| 0.001  | 0.001| 0.001  | 0.001| 0.001  | 0.001|

*p = 0.029, BAD, baseline artery diameter; BMI SDS, body mass index standard deviation score; FMD, flow-mediated dilation; HDL-C, high-density lipoprotein cholesterol; Ht SDS, height standard deviation score; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

The duration of disease should represent an important factor in the deterioration of FMD, both in adults and in adolescents. More recently, Wiltshire and colleagues studied FMD in 36 diabetic children (mean age 14 years, mean duration of T1DM < 6 years) and in 20 healthy controls. Children with T1DM without diabetic complications had attenuated endothelial function compared with controls. These results were confirmed later by Babar and colleagues. In 2010, Harrington identified aortic IMT, but not carotid IMT, as an early marker of preclinical atherosclerosis in T1DM children that correlated with the metabolic control measurement (HbA1c), age and levels of LDL. On this basis, our study illustrates data from a longitudinal evaluation of FMD in the same population of children and adolescents with T1DM. Therefore, our results not only confirm that the evaluation of endothelial dysfunction through FMD in diabetic patients could have a predictive importance to precociously individuate vascular damage, but also that diabetic pediatric patients rapidly impaired their endothelial function.

The duration of disease should represent an important factor in the development of renal damage, but also for the development and progression of heart disease, stroke, peripheral vascular disease, venous thrombosis, insulin resistance and tumor growth. Singh et al. demonstrated that the alteration of FMD precedes structural vessel abnormalities. In fact, Singh simultaneously examined both FMD and carotid intima media thickness (IMT) in diabetic adolescents and healthy teenagers, finding no differences in carotid IMT but a lower FMD in the diabetic group. The authors concluded that, although endothelial function seems to be impaired within the first decade of the onset of T1DM, an increase in carotid IMT would only occur after a considerably longer exposure to the diabetic milieu. More recently, Wiltshire and colleagues studied FMD in 36 diabetic children (mean age 14 years, mean duration of T1DM < 6 years) and in 20 healthy controls. Children with T1DM without diabetic complications had attenuated endothelial function compared with controls. These results were confirmed later by Babar and colleagues. In 2010, Harrington identified aortic IMT, but not carotid IMT, as an early marker of preclinical atherosclerosis in T1DM children that correlated with the metabolic control measurement (HbA1c), age and levels of LDL. On this basis, our study illustrates data from a longitudinal evaluation of FMD in the same population of children and adolescents with T1DM. Therefore, our results not only confirm that the evaluation of endothelial dysfunction through FMD in diabetic patients could have a predictive importance to precociously individuate vascular damage, but also that diabetic pediatric patients rapidly impaired their endothelial function.
of T1DM could cause mitochondrial alterations that may lead to long-term DNA damage and cell injury. Therefore, it is not the impairment of FMD, which is already present, but the rapidity of its deterioration that could be caused by a persistently inadequate metabolic control. The latter may play a negative synergic effect on endothelial function together with aging, physical inactivity and genetic and nutritional factors involving, in their turns, inflammatory and/or oxidative events. Our study clearly points out that a near-to-adequate glycemic control in T1DM patients is not sufficient to fully prevent the widespread vasculopathy and it also suggests that other intrinsic and extrinsic factors are involved in the evolution of a well-established vascular endothelial damage. Very recently, Peña et al.3 studied 52 T1DM children and adolescents with continuous glucose monitoring, used to evaluate glucose variability and hypoglycemia indices, and FMD. Children with T1DM had lower FMD than controls and, moreover, the authors found that hypoglycemia, but not glucose variability, related to impaired FMD in the T1DM group. Hypoglycemia may be an additional risk factor for early cardiovascular disease, but the effect of glucose variability, independent from HbA1c, on vascular function remains uncertain. Also, in 2007, the genetic susceptibility involving class II human leukocyte antigen (HLA) genes was suspected to be involved in atherogenic vascular phenotype of T1DM patients. An independent association between HLA-DQ2/8 genotype, which is known to confer a high risk for T1DM, and atherogenic lipid and vascular endothelial phenotypes was demonstrated in 86 T1DM children and adolescents.40 Moreover, environmental factors as well as folate deficiency could influence endothelial function reducing cofactors of nitric oxide synthase and acting directly on the functional activity of nitric oxide synthase. Similarly, hyperglycemia affects nitric oxide availability by causing nitric oxide degradation and uncoupling endothelial nitric oxide synthase. Folate seems to restore the function of “uncoupled” endothelial nitric oxide synthase and enhances the endothelial production of nitric oxide.41 This hypothesis seems to be supported by the results of a small Australian randomized, double-blind, placebo-controlled study, in which both high-dose folate (5 mg/daily) and high-dose vitamin B6 (100 mg/daily) normalized unpaired endothelial function in 124 children with T1DM.42 Endothelial function could also be influenced by physical activity. In fact, enhanced blood flow in arteries induces a vascular stress that results in liberation of nitric oxide during and after exercise. This mechanism could lower vascular resistance and improve FMD. Recently, the effect of physical activity on FMD was studied in T1DM children and adolescents. In one study, T1DM children who did more than 60 min per day of moderate-to-vigorous physical activity had higher FMD than inactive patients with diabetes, but not as high as active healthy subjects.43 Also a longitudinal study evaluating FMD before and after 18-week exercise training in seven children with T1DM confirmed that exercise training could improve FMD (before vs after: 7.5 ± 4.2 vs 12.4 ± 5.2%, P = 0.038) and supported a causal link between physical activity and endothelial dysfunction.44

Lipid alterations play a major role in determining the progression of atherosclerosis; so it is reasonable to suppose a correlation between lipid concentration and FMD. Recent data demonstrated better endothelial function in adults with CHD who have LDL levels <80 mg/dL, when compared with those with LDL between 80 and 100 mg/dL.44 In our study, the lipid profile apparently does not significantly influence FMD, as could be suggested by the absence of any significant correlation between both cholesterol and triglyceride levels and FMD. However, the few studies in children and adolescents with T1DM show contrasting results. While Singh et al.32 showed a significant negative correlation between FMD and both total and LDL-cholesterol, Suys et al.46 demonstrated that FMD had no correlation with LDL-cholesterol. However, in contrast with previous findings,4 it was recently shown that in preadolescent children with T1DM, the attenuated FMD measurements were coupled with systemic inflammation without a direct correlation with lipid levels.34 We suppose that other variables than lipids could be more important in FMD regulation, particularly during the early stages of blood vessel dysfunction in diabetes, like hemodynamic stress. Moreover, getting ready for further studies, the LDL-oxidation molecules and the production of advanced glycation end-products have to be considered reliable markers of the atherosclerosis process. Recently, the role of low circulating endothelial progenitors cells (EPC), a new biomarker of cardiovascular risk in adults, was investigated in 52 T1DM children: an increased frequency of EPC observed in children with T1DM, probably resulting from their mobilization in order to repair damaged endothelium, was negatively correlated with endothelial function.47 These fields have to be now considered the direction of future studies.

Regarding the differences of endothelial dysfunction between male and female subjects, our results confirm data already reported in the literature.48–50 In general, women have a vasomotor activity (mediated of endothelium) greater than that of men of the same age. There are many hypotheses about the reason why this happens. First of all, women have less body surface area, a lower BMI and a lower arterial diameter. Mizia-Stec et al. have already demonstrated that the brachial artery diameter correlates well with the amount of FMD%.39 This is the crucial point that may explain the observed differences. In our case the diameter of the brachial artery is significantly different in boys and girls, even if at follow up girls present a higher BMI SDS than boys. Moreover, Spearman’s analysis does not document a correlation between FMD and BAD either at the beginning or at the end of the study. Therefore, it seems improbable that changes in BAD could significantly influence FMD alterations. Others factors that regulate vascular vasodilation have to be taken into account, such as puberty age. At this stage of life, women acquire the ability to produce large amounts of estrogen, which are known as agents that can improve endothelial function.48 The mechanisms of endothelial dysfunction and accelerated atherosclerosis in diabetes are multifactorial and have not been fully characterized yet.

In conclusion, the most impressive results from our study are those related to the dramatic impairment of the FMD after a relatively short follow up, especially in boys. Being male is the unique predictor of FMD worsening demonstrated by our study. Because endothelial dysfunction appears earlier (than expected), children with T1DM are at high risk to develop atherosclerosis.
This impairment of endothelial function, unpredictable on the basis of glycemic control, circulating lipid concentrations and blood pressure values, allows us to assume a more aggressive treatment of T1DM. Cardiac care for pediatric patients with T1DM should now be focused on aggressive management of traditional cardiovascular risk factors, to optimize the well-recognized ones as well as the new specific risk factors nowadays becoming available, like FMD.

However, our study presents several limitations. Therefore, our results require a careful interpretation. First of all, we included a small number of patients. In addition to it, our analysis does not include serum insulin levels that could alter FMD-inducing vasodilatation, even though our patients are tested when fasted, before administration of morning insulin dose. Another methodological limitation is the choice of FMD with its technical and interpretative limits. For example, FMD is operator-dependent and, therefore, it is not suitable for multicenter study. As is known, BAD influences FMD percentage change and, for any given absolute change in the post-flow stimulus diameter, a larger baseline diameter yields a smaller measure of percentage change and smaller arteries appear to dilate relatively more than larger arteries do. Nevertheless, in our study, no correlation between FMD and BAD has been documented and it allows us to suppose that the structural change of BAD is not the main determining factor of FMD. Moreover, despite the fact that the evaluation of FMD can be influenced by several factors (temperature, diet, menstrual cycle, technicalities) changing day to day with consequent variable results, Sørensen has already demonstrated that the long-term within-subject variation during weeks and months is acceptable and its measurement is reproducible.

In conclusion, we demonstrated that in a few months, endothelial function severely impairs in children and adolescents with T1DM. We think that the ultrasound assessment of FMD might be a useful tool for cardiovascular risk stratification of pediatric patients with T1DM, at least in a research setting.

In the approach to children and adolescents with T1DM, however, it is time to consider surrogate methods to examine endothelial function, in order to better understand the impact of the treatment on the vascular status. Moreover, further studies are required to individuate both factors involved in the impairment of FMD, and therapeutic interventions with beneficial effects on endothelial function.

References

1. Järvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation* 2004; 109: 1750–5.
2. Nathan DM. Long-term complications of diabetes mellitus. *N. Engl. J. Med.* 1993; 328: 1676–85.
3. Peña AS, Couper JJ, Harrington J et al. Hypoglycemia, but not glucose variability, relates to vascular function in children with type 1 diabetes. *Diabetes Technol. Ther.* 2012; 14: 457–62.
4. Jarvisalo MJ, Putto-Laurila A, Jartti L et al. Carotid artery intima-media thickness in children with type 1 diabetes. *Diabetes* 2002; 51: 493–8.
5. Orchard TJ, Costacou T, Kretowski A, Nesto RW. Type 1 diabetes and coronary artery disease. *Diabetes Care* 2006; 29: 2528–39.
6. Celermajer DS, Sorensen KE, Gooch VM et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992; 340: 1111–5.
7. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; 115: 1285–95.
8. Bruyndonckx L, Hoymans VY, Van Craenbroeck AH et al. Assessment of endothelial dysfunction in childhood obesity and clinical use. *Oxid. Med. Cell. Longev.* 2013. doi: 10.1155/2013/174782.
9. Ebara T, Conde K, Kako Y et al. Delayed catabolism of apoB-48 lipoproteins due to decreased heparin sulfate proteoglycan production in diabetic mice. *J. Clin. Invest.* 2000; 105: 1807–18.
10. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute, NIH Publication 01-3670, 2001.
11. Kavey REW, Allada V, Daniels SR et al. Cardiovascular risk reduction in high-risk pediatric patients. *Circulation* 2006; 114: 2710–38.
12. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 2002; 287: 2563–9.
13. Nathan DM, Cleary PA, Backlund JY et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N. Engl. J. Med.* 2005; 353: 2643–53.
14. Diabetes Control and Complications Trial Research Group. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. *J. Pediatr.* 1994; 125: 177–88.
15. Danne T, Mortensen HB, Hougaard P et al. Persistent differences among centers over 3 years in glycemic control and hypoglycaemia in a study of 3805 children and adolescents with type 1 diabetes from the Hvidore Study Group. *Diabetes Care* 2001; 24: 1342–7.
16. Mortensen HB, Hougaard P. Comparison of metabolic control in a cross-sectional study of 2873 children and adolescents with IDDM from 18 countries. The Hvidore Study Group on Childhood Diabetes. *Diabetes Care* 1997; 20: 714–20.
17. Springer D, Dziura J, Tamborlane WV et al. Optimal control of type 1 diabetes mellitus in youth receiving intensive treatment. *J. Pediatr.* 2006; 149: 227–32.
18. Silverstein JH, Klingensmith G, Copeland K et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. *Diabetes Care* 2005; 28: 186–212.
19. Mahmud F, Earing MG, Lee RA, Lteif AN, Driscoll DJ, Lerman A. Altered endothelial function in asymptomatic male adolescents with type 1 diabetes. *Congenit. Heart Dis.* 2006; 1: 98–103.
20. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch. Dis. Child.* 1969; 44: 291–303.
21. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 2010; 33: S11–S61.
22. Sørensen KE, Celermajer DS, Spiegelhalter DJ et al. Non-invasive measurement of human endothelium dependent responses: accuracy and reproducibility. *Br. Heart J.* 1995; 74: 247–53.
23. Corretti MC, Anderson TJ, Benjamin EJ et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol.* 2002; 39: 257–65.
24. Rossi R, Nuzzo A, Origigliani G, Modena MG. Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women. *J. Am. Coll. Cardiol.* 2008; 51: 997–1002.
Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N. Engl. J. Med.* 1998; **338**: 1650–6.

Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study. *Circulation* 2001; **104**: 2815–9.

Choy YH, Couper JJ, Donaghy KC. Complications of childhood diabetes and the role of technology. *Pediatr. Endocrinol. Rev.* 2010; **7** (S3): 422–31.

Giannini C, Mohn A, Chiarelli F, Kelnar CJ. Macrovascular angiopathy in children and adolescents with type 1 diabetes. *Diabetes Metab. Res. Rev.* 2011; **27**: 436–60.

Donaghy KC, Robinson J, McCredie R, Fung A, Silink M, Cè GV, Rohde LE, da Silva AM, Punales MK, de Castro AC, Harrington J, Pena AS, Gent R, Hirte C, Piotto L, Couper JJ. Aortic intima-media thickness in children with insulin-dependent diabetes. *Diabetes Care* 2011; **34**: 681–5.

Harrington J, Penn AS, Gent R, Hirte C, Couper J. Aortic intima-media thickness is an early marker of atherosclerosis in children with type 1 diabetes mellitus. *J. Am. Coll. Cardiol.* 2003; **41**: 661–5.

Wiltshire EJ, Gent R, Hirte C, Penn A, Thomas DW, Couper JJ. Endothelial dysfunction relates to folate status in children and adolescents with type 1 diabetes. *Diabetes* 2002; **51**: 2282–6.

Babar GS, Zidan H, Widlansky ME et al. Impaired endothelial function in preadolescent children with type 1 diabetes. *Diabetes Care* 2011; **34**: 681–5.

Ceriello A, Ihnat MA, Thorpe JE. Clinical review 2: the “metabolic memory”: is more than just tight glucose control necessary to prevent diabetic complications? *J. Clin. Endocrinol. Metab.* 2009; **94**: 410–5.

Odermarsky M, Nilsson A, Lernmark A, Sjoblad S, Liuba P, Athrogenic vascular and lipid phenotypes in young patients with type 1 diabetes are associated with diabetes high-risk HLA genotype. *Am. J. Physiol. Heart Circ. Physiol.* 2007; **293**: H3175–H3179.

Stroes SE, van Faassen EE, Yo M et al. Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circ. Res.* 2000; **86**: 1129–34.

MacKenzie KE, Wiltshire EJ, Gent R, Hirte C, Piotto L, Couper JJ. Folate and vitamin B6 rapidly normalize endothelial dysfunction in children with type 1 diabetes mellitus. *Pediatrics* 2006; **118** (1): 242–53.

Trigona B, Aggoun Y, Maggio A et al. Preclinical noninvasive markers of atherosclerosis in children and adolescents with type 1 diabetes are influenced by physical activity. *J. Pediatr.* 2010; **157**: 533–9.

Seeger JPH, Thijssen DHJ, Noordam K, Cranen MEC, Hopman MT, Nijhuis-van der Sanden MW. Exercise training improves physical fitness and vascular function in children with type 1 diabetes. *Diabetes Obes. Metab.* 2011; **13**: 382–4.

Kuvin JT, Patel AR, Slinsky KA, Pandian NG, Karas RH. Comparison of flow-mediated dilatation of the brachial artery in coronary patients with low-density lipoprotein cholesterol levels <80 mg/dl versus patients with levels 80 to 100 mg/dl. *Am. J. Cardiol.* 2005; **95**: 93–5.

Suy S, de Beeck LO, Rooman R et al. Impact of oxidative stress on the endothelial dysfunction of children and adolescents with type 1 diabetes mellitus: protection by superoxide dismutase? *Pediatr. Res.* 2007; **62**: 456–61.

Glowinska-Olszewska B, Moniuszko M, Hryniewicz A et al. Relationship between circulating endothelial progenitor cells (EPCs) and endothelial dysfunction in children with type 1 diabetes – a novel paradigm of early atherosclerosis in high-risk young patients. *Eur. J. Endocrinol.* 2013; **168**: 153–61.

Mamhud FH, Earing MG, Lee RA, Lteif AN, Driscoll DJ, Lerman A. Altered endothelial function in asymptomatic male adolescents with type 1 diabetes. *Congenit. Heart Dis.* 2006; **1**: 98–103.

Mizia-Stec K, Zbigniew G, Mizia M et al. Flow-mediated dilation and gender in patients with coronary artery disease: arterial size influences gender differences in flow-mediated dilation. *Echocardiography* 2007; **24**: 1051–7.

Marra G, Cotroneo P, Pitocco D et al. Early increase of oxidative stress and reduced antioxidant defenses in patients with uncomplicated type 1 diabetes: a case for gender difference. *Diabetes Care* 2002; **25**: 370–5.

Jarvisalo MJ, Raitakari OT. Ultrasound assessment of endothelial function in children. *Vasc. Health Risk Manag.* 2005; **1**: 227–33.

McNeal CJ, Wilson DP, Christou D et al. The use of surrogate vascular markers in youth at risk for premature cardiovascular disease. *J. Pediatr. Endocrinol. Metab.* 2009; **22**: 195–211.

Rogers M, Vogel R, Criqui M, Harrington D, Lima J, Roman M. 34th Bethesda Conference: task force #3 – what is the spectrum of current and emerging techniques for the noninvasive measurement of atherosclerosis? *J. Am. Coll. Cardiol.* 2003; **41**: 1886–98.