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Comparison of brachial artery flow-mediated dilation in youth with type 1 and type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Brachial artery flow-mediated dilation (FMD) is a method of evaluating the function of vascular endothelial cells and is utilized for early diagnosis of atherosclerotic diseases. Only a few studies evaluated the risks for major vascular complications in youth with type 1 and 2 diabetes mellitus from the aspect of the early development of atherosclerosis. We studied whether there is a difference in vascular endothelial cell function between youth with type 1 and 2 diabetes mellitus.

Materials and Methods: We assessed %FMD of 24 patients with type 1 diabetes mellitus and 27 patients with type 2 diabetes mellitus aged 12–20 years along with glycated hemoglobin, lipid metabolism markers such as triglycerides, and inflammatory biomarkers such as total adiponectin levels in adolescent patients with type 1 or 2 diabetes mellitus. The significance of the difference in each factor between the type 1 and type 2 diabetes groups was assessed using Student's *t*-test.

Results: The %FMD was significantly lower in patients with type 2 diabetes. The body mass index and blood pressure were significantly higher, and total and high-molecular-weight adiponectin levels were significantly lower in patients with type 2 diabetes. %FMD significantly correlated with systolic blood pressure.

Conclusions: The results suggest that youth with type 2 diabetes have more advanced damage of the vascular endothelium and therefore are at higher risk for major vascular complications. Therefore, monitoring the progression of atherosclerosis would also be beneficial in youth with diabetes mellitus, and measurement of FMD could be further warranted.

INTRODUCTION

Various methods of non-invasively assessing the degree of atherosclerosis have been developed. Brachial artery flow-mediated dilation (FMD) has been reported to be effective for early detection of atherosclerotic lesions[1]. FMD evaluates the function of vascular endothelial cells, and reduced %FMD is a predictive factor for major vascular complications including cardiovascular diseases[2,3]. The %FMD as an independent predictor of long-term cardiovascular events was reported to be less than 8.1 in adults[4]. Adults with type 1 and type 2 diabetes were reported to have decreased %FMD[5]. Decreased %FMD is also found in diabetic youth, suggesting that diabetes also causes dysfunction of vascular endothelial cells in young diabetics, and would lead to early atherosclerotic changes[6–8].

It is well known that diabetes mellitus is a risk factor for macrovascular complications in adults. It is reported that the long-term prognosis is poor with respect to microvascular complications in youth type 2 diabetes compared with youth type 1 diabetes[9,10]. However, few studies have evaluated the incidence of major vascular complications in youth with type 1 and type 2 diabetes from the standpoint of early atherosclerosis[11].

Therefore, we measured FMD, along with several other important parameters for determining progression of atherosclerosis, such as lipid profiles and biomarkers of inflammation, and examined whether those markers are significantly different between youth with type 1 and type 2 diabetes.

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MATERIALS AND METHODS

Study Participants

A total of 24 patients with type 1 diabetes mellitus (11 males and 13 females; age range 12–20 years) and 27 patients with type 2 diabetes mellitus (15 males and 12 females; age range 12–20 years), who visited the Yokohama City University Medical Center (Kanagawa, Japan) between January and December 2011 were included in the present study. All patients had reached puberty. Diabetes was diagnosed according to the Classification and Diagnosis of Diabetes Mellitus by the Japan Diabetes Society[12]. The types of diabetes were classified according to the mode of onset, tendency to ketosis, blood C-peptide value and the islet-associated antibodies. Patients with secondary diabetes and unclassifiable diabetes were excluded. Patients classified as type 1 diabetes were 85% positive in either glutamate decarboxylase (GAD) antibody or insulinoma-associated antigen-2 (IA-2) antibody or both. Patients classified as type 2 diabetes were all negative in both GAD antibody and IA-2 antibody. Patients' baseline characteristics (age, disease duration, body mass index [BMI] = bodyweight [kg] / height [cm]^2, and systolic and diastolic blood pressure) and data from blood chemistry (glycated hemoglobin [HbA1c]; triglyceride; total, low-density lipoprotein [LDL] cholesterol and high-density lipoprotein [HDL] cholesterol levels; high-sensitivity C-reactive protein [CRP]; and total and high-molecular weight adiponectin) were collected and compared between the two groups. The protocol of the present study was approved by the institutional research ethics committee (approval number: D120524002, approved on 24 May 2012). Written informed consent was obtained from the guardians of all of the participating patients.

Measurement of FMD

Flow-mediated dilation was measured using an ultrasound system (model HD25XE; Philips, Bothell, WA, USA) with a 10-MHz linear transducer. All of the measurements were carried out by a single physician to minimize interperson variability. After resting in the supine position for more than 5 min, the resting end-diastolic diameter of the brachial artery was measured with the transducer applied parallel to the artery. Next, blood was expulsed from the brachial artery for 5 min with a sphygmomanometer cuff placed at the forearm and inflated at 200 mmHg, and another measurement of end-diastolic brachial artery diameter was obtained 60 s after the cuff was deflated[13]. %FMD was calculated as the percentage change in the arterial diameter before and after expulsion.

Blood Chemistry

A blood sample was drawn in the fasting state on the same day that FMD was measured. Levels of total and high-molecular weight adiponectin; high-sensitivity CRP; total, LDL and HDL cholesterol; and triglyceride were measured at SRL Inc. (Hachioji, Japan). Specifically, serum total and high-molecular weight adiponectin levels were measured by latex immunonephelometry and chemiluminescent enzyme immunoassay (CLEIA), respectively. The high-sensitivity CRP level was determined by latex immunonephelometry. Total cholesterol was measured by the cholesterol dehydrogenase method, and triglyceride level was measured by the enzyme method. LDL and HDL cholesterol levels in serum were directly measured. HbA1c level was measured by high-performance liquid chromatography (Hi AUTO, HA8150; Arkray, Kyoto, Japan). HbA1c values were converted from Japan Diabetes Society to National Glycohemoglobin Standardization Program values[14,15].

Statistical Analyses

Data were statistically analyzed using SPSS version 13 (SPSS, Chicago, IL, USA). For comparison of background characteristics and results of blood chemistry tests, Student's *t*-test was carried out. The relationship between the %FMD and clinical variables was analyzed using a linear regression. Multiple regression analysis was carried out to identify the independent risk factors with the use of factors identified in univariate analyses. $P < 0.05$ was considered statistically significant. The following parameters were considered to be normally distributed and raw numbers were used in the statistical analyses: patients' age and duration of diabetes, HbA1c, systolic and diastolic blood pressure, and %FMD. The other data (BMI, total cholesterol, LDL and HDL cholesterol, triglyceride, high-sensitivity CRP, and total and high-molecular-weight adiponectin) were logarithmically transformed to obtain a normal distribution.

RESULTS

The patients' characteristics and data from blood chemistry tests in the type 1 and type 2 diabetes groups are summarized in Table 1. The age range was similar in the two groups. The duration of diabetes was significantly shorter in the type 2 diabetes group than in the type 1 diabetes group (6.0 ± 2.8 vs 3.6 ± 2.7, $P < 0.01$). The BMI, systolic blood pressure and diastolic blood pressure were significantly higher, and the total and high-molecular weight adiponectin and HDL cholesterol levels were significantly lower in the type 2 diabetes group than in the type 1 diabetes group. The HbA1c levels were similar in the two groups.

The %FMD was significantly lower in the type 2 diabetes group than in the type 1 diabetes group (7.9 ± 4.0 vs 10.5 ± 5.1, $P < 0.05$). There was no significant difference in % FMD when comparison was made in each sex (Figure 1).

Overall, %FMD significantly correlated with sex, BMI, systolic blood pressure, HDL cholesterol, total adiponectin, high-molecular weight adiponectin and high-sensitivity CRP in univariate analysis. Multiple regression analysis was carried out with the significant factors shown in the univariate analyses. % FMD significantly correlated with systolic blood pressure in multivariate analysis (Table 2). In the type 1 diabetes group, %FMD significantly correlated with systolic blood pressure in

Table 1 | Clinical characteristics and chemical data of the study participants according to the type of diabetes

	Type 1 diabetes	Type 2 diabetes	*P*
n	24	27	
Sex (male/female)	11/13	15/12	0.50
Age (years)	16.6 ± 2.0	17.1 ± 2.0	0.32
Duration of diabetes (years)	6.0 ± 2.8	3.6 ± 2.7	<0.01
Height (cm)	164.1 ± 7.9	163.0 ± 11.0	0.66
BMI (kg/cm^2)	21.7 ± 2.2	27.8 ± 5.2	<0.001
SBP (mmHg)	114.8 ± 9.9	124.6 ± 15.7	<0.01
DBP (mmHg)	63.2 ± 8.8	72.0 ± 12.3	<0.01
HbA1c (%)	7.8 ± 1.4	7.6 ± 2.0	0.66
HDL-C (mg/dL)	67.7 ± 9.7	49.3 ± 9.9	<0.001
Total-C (mg/dL)	174.5 ± 37.6	176.3 ± 32.2	0.80
Triglycerides(mg/dL)	79.0 ± 62.3	119.5 ± 84.3	0.01
LDL-C (mg/dL)	91.4 ± 29.1	98.5 ± 25.7	0.31
hs-CRP (ng/mL)	567 ± 1,016	2,188 ± 6,251	<0.01
Adiponectin (μg/mL)	13.5 ± 6.7	7.0 ± 4.3	0.000
HMW adiponectin (μg/mL)	6.1 ± 3.1	2.8 ± 2.3	0.000
Insulin	24	1	
Metformin	0	8	
Insulin + metformin	0	2	
Other antidiabetic drugs	0	4	
Antihypertensive drug (ACE-I)	0	3	
Statin	0	2	

The significance of the difference in each factor between the type 1 and type 2 diabetes groups was assessed using Student's *t*-test. Levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), high-sensitivity C-reactive protein (CRP) and high-molecular weight adiponectin were logarithmically transformed and considered to be normally distributed. ACE-I, angiotensin-converting enzyme inhibitor; BMI, body mass index; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; HMW adiponectin, high-molecular weight adiponectin; hs-CRP, high sensitivity C-reactive protein; SBP, systolic blood pressure; Total-C, total cholesterol.

univariate analysis (Table 3). In the type 2 diabetes group, % FMD significantly correlated with the sex, BMI, systolic blood pressure, total adiponectin, high-molecular-weight adiponectin and high-sensitivity CRP in univariate analysis. Multiple regression analysis was carried out with the significant factors shown in the univariate analyses. %FMD did not significantly correlate with any factor in multivariate analysis (Table 4).

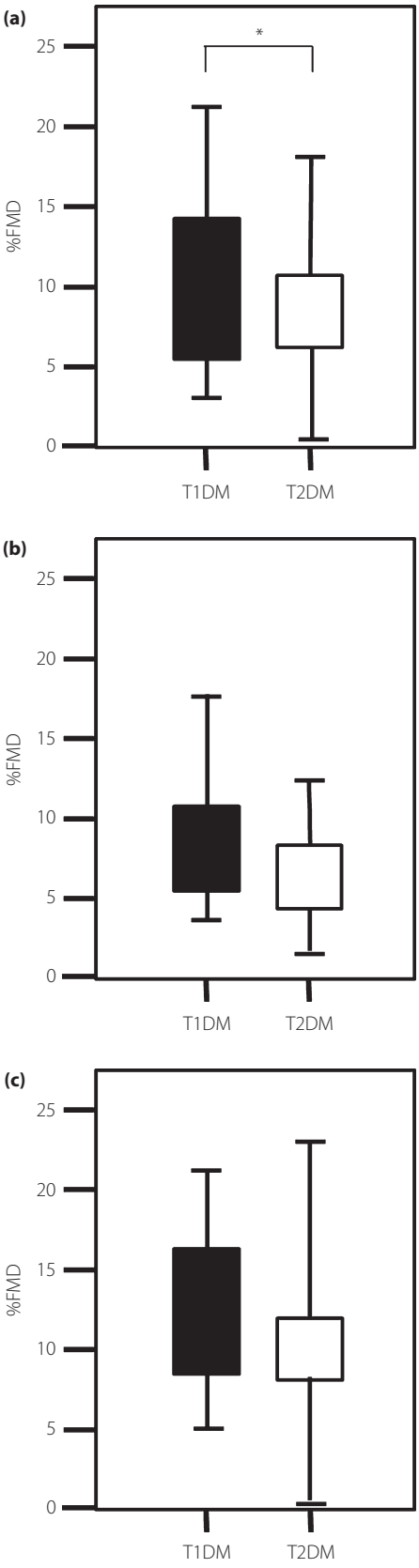

Figure 1 | Flow-mediated dilation (FMD) percentages of the type 1 (T1DM) and type 2 diabetes (T2DM) groups in (a) all patients, (b) men, and (c) women. %FMD was calculated as the percentage change of FMD from the baseline value to that after 5 min expulsion. Boxes indicate the 25th percentile and 75th percentile, and bars indicate the maximum and minimum values. *$P < 0.05$, Student's *t*-test.

Table 2 | Linear regression of percentage of flow-mediated dilation with clinical characteristics and chemical data in both patients with type 1 and type 2 diabetes

	Univariate analysis		Multivariate analysis	
	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*
Type of diabetes	−2.57 (−5.13 to −0.01)	0.05		
Sex	−4.21 (−6.59 to −1.85)	<0.01	−1.93 (−4.39 to 0.51)	0.12
Statin usage	−0.59 (−7.44 to 6.26)	0.86		
Duration of diabetes	0.31 (−0.13 to 0.74)	0.17		
BMI	−0.41 (−0.68 to −0.15)	<0.01	0.12 (−0.23 to 0.47)	0.51
HbA1c	−0.20 (−0.95 to 0.55)	0.59		
SBP	−0.18 (−0.26 to −0.11)	<0.001	−0.15 (−0.25 to −0.05)	<0.01
DBP	−0.09 (−0.20 to 0.02)	0.11		
HDL-C*	18.1 (6.68 to 29.6)	<0.01	2.29 (−10.5 to 15.0)	0.71
Total-C *	3.67 (−12.6 to 19.9)	0.65		
Triglycerides*	−3.48 (−8.05 to 1.08)	0.13		
LDL-C*	0.49 (−9.10 to 10.1)	0.92		
Adiponectin*	6.03 (1.49 to 8.52)	0.01	−14.5 (−36.9 to 7.95)	0.20
HMW adiponectin*	5.09 (1.66 to 8.52)	<0.01	13.5 (−4.19 to 31.2)	0.13
hs-CRP*	−2.48 (−4.6 to −0.39)	0.02	−0.89 (−2.88 to 1.10)	0.37

Sample size for regression: $n = 51$. *Correlation with log transformation. BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HMW adiponectin, high-molecular weight adiponectin; hs-CRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; Total-C, total cholesterol.

Table 3 | Linear regression of percentage of flow-mediated dilation with clinical characteristics and chemical data in patients with type 1 diabetes

	Univariate analysis	
	Coefficient (95% CI)	*P*
Sex	−3.92 (−7.84 to 0.00)	0.05
Duration of diabetes	0.27 (−0.47 to 1.02)	0.46
BMI	−0.08 (−1.21 to 0.85)	0.71
HbA1c	−0.37 (−1.92 to 1.19)	0.63
SBP	−0.27 (−0.45 to −0.08)	<0.01
DBP	−0.12 (−0.35 to 0.12)	0.31
HDL-C*	30.4 (−1.59 to 62.5)	0.06
Total-C *	9.65 (−15.7 to 35.0)	0.44
Triglycerides*	1.89 (−6.16 to 9.95)	0.63
LDL-C*	2.27 (−12.2 to 16.8)	0.75
Adiponectin*	1.99 (−9.70 to 13.7)	0.73
HMW adiponectin*	2.68 (−7.67 to 13.0)	0.60
hs-CRP*	−0.93 (−5.36 to 3.49)	0.67

Sample size for regression: $n = 24$. *Correlation with log transformation. BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HMW adiponectin, high-molecular weight adiponectin; hs-CRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; Total-C, total cholesterol.

DISCUSSION

There are a variety of methods of non-invasively examining atherosclerosis. Measurement of FMD is one such method, and its usefulness in detecting early atherosclerosis has been reported[1]. FMD evaluates the function of vascular endothelial cells[2,3]. Comparison of intima media thickness (IMT) and FMD by ultrasonography in obese children and their age-matched healthy control children showed that there was a significant difference only in FMD[16]. Therefore, it has been considered that FMD is a more appropriate marker than IMT to evaluate arteriosclerosis at the early stage in obese children. It is suggested that in Japanese healthy youth under the age of 20 years the normal range of %FMD mild reduction value is 8.0 or less and the significant reduction value is 6.0 or less[17].

Macroangiopathy and microangiopathy are vascular complications of type 1 and type 2 diabetes. These vascular complications are involved in the onset of glucose intolerance from the early stage through endothelial dysfunction. Vascular complications result from the early stage of vascular endothelial cell dysfunctions in both patients with type 1 and type 2 diabetes[5,18]. A decrease in %FMD and vascular endothelial cell dysfunction are found even in youth with diabetes, and contribute to early atherosclerotic changes[6–8].

Increased production of advanced glycosylation end-products[19,20], decreased functional endothelial nitric oxide synthase[21], increased tissue superoxide dismutase[22–24], enhanced signals for inflammation and lipid metabolism[25], activation of polyol cascade[26] and effects of epigenetics[27,28] have all been suggested to cause vascular endothelial cell dysfunction in patients with high serum glucose.

Dysfunction of vascular endothelial cells can also be caused by various other factors, such as obesity, hypertension, lipid abnormality, lack of exercise, smoking, aging and menopause[5,29,30]. In obese patients, abnormal regulation of adipocytokine, such as low serum adiponectin levels, occurs as a result of adipocyte dysfunction that has been caused by an accumulation of visceral fat. Abnormalities of blood pressure and lipid metabolism are also interrelated. These changes could further accelerate atherosclerosis, leading to major vascular damages, such as cardiovascular and cerebrovascular diseases[31].

In the present study, we showed that the %FMD was significantly lower in the type 2 diabetes group than in the type 1 diabetes group. Blood glucose control, as evaluated by the

Table 4 | Linear regression of percentage of flow-mediated dilation with clinical characteristics and chemical data in patients with type 2 diabetes

	Univariate analysis		Multivariate analysis	
	Coefficient (95%CI)	*P*	Coefficient (95% CI)	*P*
Sex	4.08 (–7.02 to –1.14)	<0.01	–2.15 (–5.14 to 0.84)	0.15
Statin usage	0.69 (–5.73 to 7.11)	0.83		
Duration of diabetes	–0.02 (–0.69 to 0.66)	0.96		
BMI	–0.42 (–0.75 to 0.10)	0.01	–0.06 (–0.42 to 0.30)	0.73
HbA1c	–0.19 (–1.01 to 0.62)	0.63		
SBP	–0.14 (–0.23 to –0.06)	<0.01	–0.09 (–0.20 to 0.01)	0.09
DBP	–0.04 (–0.17 to 0.10)	0.57		
HDL-C*	13.0 (–4.34 to 30.3)	0.14		
Total-C *	–2.2 (–23.1 to 18.7)	0.83		
Triglycerides*	–5.66 (–11.4 to 0.07)	0.06		
LDL-C*	0.74 (–12.3 to 13.8)	0.91		
Adiponectin*	6.52 (0.74 to 9.71)	0.04	–7.56 (–33.7 to 18.6)	0.55
HMW adiponectin*	5.22 (0.74 to 9.71)	0.02	8.39 (–11.0 to 27.8)	0.38
hs-CRP*	–2.49 (–5.08 to 0.10)	0.05	–1.57 (–3.97 to 0.38)	0.19

Sample size for regression: $n = 27$. *Correlation with log transformation. BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HMW adiponectin, high-molecular weight adiponectin; hs-CRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; Total-C, total cholesterol.

HbA1c level, was equivalent in the two groups at the time of assessment of FMD. These data suggest that dysfunction of vascular endothelial cells is more severe in patients with type 2 diabetes than in those with type 1 diabetes. There were many patients with a high BMI accompanied by being overweight or obese in the type 2 diabetes group. Also, the adiponectin and high-molecular-weight adiponectin levels were significantly lower in the type 2 diabetes group, indicating excess fat accumulation, particularly in patients with type 2 diabetes. Obesity associated with type 2 diabetes triggers not only insulin resistance, but hypertension, abnormal lipid metabolism, decreased adiponectin production and chronic systemic inflammation. All of these factors could further accelerate atherosclerosis, leading to major vascular damages. Systolic blood pressure was significantly correlated with %FMD in both the type 1 and type 2 diabetes groups. Although it is well known that the risk of major vascular complication is considerably increased in diabetic patients with hypertension, an elevated blood pressure might directly cause dysfunction of vascular endothelial cells in these patients.

In youth with type 2 diabetes, insulin resistance associated with obesity is considered the main factor for the pathogenesis[32]. Essentially, type 2 diabetes is a complicated disease consisting of a variety of factors, and its pathogenesis is not uniform. Ideally, comparison with the healthy control group is necessary, but it was difficult to select age-matched healthy control participants in daily pediatric practice at the outpatient clinic. Then, patients with type 1 diabetes, which was frequently found in childhood and is a risk factor for cardiovascular complications[33,34], were selected as the control group. In the present study, the case number was small, and there was no significant difference in %FMD when comparison was made in each sex and obesity-degree group. Investigation in a larger number of cases and comparison with a control group will be necessary.

The present data suggest that, even in youth, dysfunction of vascular endothelial cells is more severe in patients with type 2 diabetes than in those with type 1 diabetes. As younger patients have longer lifespans, early detection of and a therapeutic approach, such as early treatment of hypertension with an angiotensin-converting enzyme inhibitor for vascular damages and atherosclerosis, are essential to prevent major vascular complications during the remaining lifespan of youth with type 2 diabetes.

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