

# Endothelium-Dependent Brachial Artery Flow Mediated Vasodilatation in Patients with Diabetes Mellitus With and Without Coronary Artery Disease

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## Abstract

**Aim of the Study :** Endothelial function as assessed by brachial artery flow mediated vasodilatation (FMD) has been shown to be impaired in patients with coronary artery disease (CAD). Since diabetes mellitus (DM) has been considered to be CAD risk-equivalent, we sought to determine whether endothelial dysfunction is present in patients with DM independent of presence of CAD.

**Methodology :** One hundred and ninety eight individuals were included in the study and divided into four groups: Group 1 - patients with risk factors for CAD, but no DM or CAD; Group 2 - patients with DM but no CAD; Group 3 - patients with CAD but no DM and Group 4 - patients with both DM and CAD. Brachial artery FMD assessment was performed once in all subjects and FMD was calculated as percentage increase in brachial artery diameter in response to increase in brachial artery flow.

**Results :** Mean FMD was significantly higher in Group 1 ( $7.03 \pm 2.87\%$ ) compared to the other three groups. Mean FMD in Group 2 ( $5.51 \pm 2.12\%$ ) was similar to that in Group 3 ( $4.56 \pm 2.70\%$ ; p value 0.195) but significantly higher than that in Group 4 ( $4.26 \pm 1.93\%$ ; p value 0.038). There was no statistically significant difference in mean FMD in Group 3 and Group 4 (p value 0.65).

**Conclusion :** Endothelial function as assessed by FMD is significantly impaired in diabetics compared to non-diabetics in absence of CAD. In addition, similar degree of impairment in endothelial function is seen in diabetics without CAD and non-diabetic patients having CAD, implying CAD risk-equivalence of diabetes.

## INTRODUCTION

Endothelial dysfunction is a key early event in atherogenesis and is known to appear long before the formation of structural atherosclerotic changes.<sup>1,2</sup> Assessment of endothelial function, thus, can provide valuable insight into pre-intrusive phase of atherosclerosis and can be used an early marker of future atherosclerotic disease. However, invasive nature of the earlier available tests for endothelial function assessment precluded their use in clinical practice.<sup>3</sup> Development of non-invasive method of endothelial function assessment by brachial artery flow mediated vasodilatation (FMD), as described by Celermajer,<sup>4</sup> provided an extremely useful tool for cardiovascular research and for clinical application. The test can be performed easily and has proven reproducibility.<sup>5</sup> International Task Force on Brachial Artery Reactivity has recently laid guidelines for performance of

FMD,<sup>6</sup> thus standardizing the test for wider application.

Several studies have demonstrated endothelial dysfunction not only in patients with established coronary artery disease (CAD),<sup>7,8</sup> but also in patients with cardiovascular risk factors including diabetes mellitus (DM).<sup>2,9-12</sup> Since diabetes is now considered to be 'CAD' risk equivalent,<sup>13</sup> we aimed to determine whether similar degree of impairment of endothelial function occurs in patients with DM and CAD that may be responsible for this 'risk equivalence'.

## METHODS

One hundred and ninety eight individuals were included in the study and divided into four groups. Groups 1 (n = 67) included subjects with risk factors for CAD, but no DM or CAD; Group 2 (n = 38) included patients with DM but no CAD; Group 3 (n = 58) included patients with CAD but no DM; and Group 4 (n = 35) included patients having both CAD and DM. Subjects in Group 1 and 2 were randomly selected from patients attending cardiology OPD of the Institute for routine checkup with no symptoms or signs of CAD. A negative treadmill test was used to confirm the

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absence of significant CAD in these individuals. Subjects in Group 3 and 4 were recruited from inpatient and outpatient departments of the institute. Diagnosis of CAD in them was established by presence of history of documented myocardial infarction or finding of critical stenosis in at least one epicardial coronary artery on coronary angiogram. Diabetes mellitus in the present study was defined as a fasting blood glucose level of  $\geq 126$  mg/dl or non-fasting blood glucose level  $\geq 200$  mg/dl or pharmacological treatment for diabetes.

Patients with history of myocardial infarction or acute coronary syndrome during preceding four weeks, history of prior coronary artery bypass grafting or percutaneous transluminal coronary angioplasty and patients admitted with overt congestive heart failure or any acute illness were excluded from the study.

Clinical evaluation, electrocardiogram, biochemical tests and assessment of brachial artery flow-mediated vasodilatation were done in all subjects after taking informed consent for the study. Treadmill test and coronary angiography were performed in selected individuals. Clinical examination included blood pressure recording, assessment of cardiovascular status and height and body weight measurements. Biochemical assessment included fasting and post-prandial blood sugar and fasting lipid profile. Patients were evaluated for presence and duration of conventional cardiovascular risk factors viz. hypertension, family history of premature CAD, dyslipidemia, current smoking and diabetes mellitus. Hypertension was defined as systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mm Hg or self reported use of anti-hypertensive medications. Dyslipidemia was defined as LDL level  $\geq 130$  mg/dl, or HDL  $\leq 40$  mg/dl, or TG  $\geq 200$  mg/dl. Family history was coded as positive if a first degree relative had a coronary event before the age of 55 years in males and 65 years in female relatives. Current smoking or tobacco use in any form was considered to be a conventional risk factor.

The brachial artery FMD assessment was performed once in all subjects using 7.5 MHz phased array linear transducer attached to HP Sonos 5500 echocardiography machine. The test was done after overnight fasting. Smoking was prohibited for at least four hours before the test and all the vasoactive drugs such as nitrates were withheld for 48 hours preceding the test. The sphygmomanometer cuff was tied in the right arm with the patient in supine position. The brachial artery was imaged in the ante-cubital fossa and its diameter

measured at end-systole. The systolic and diastolic VTIs (velocity time integrals) were also recorded using pulsed wave Doppler. The arm was then occluded with the sphygmomanometer cuff inflated to at least 50 mm Hg above systolic blood pressure for five minutes. The same measurements were repeated immediately (within 15 seconds) after release of the cuff. The brachial artery diameter was measured again at 1 minute to assess FMD. The ultrasound of the brachial artery was continuously recorded on the videotape before, during and upto two minutes after release of occlusion. The still images were also recorded on digital Enconcert system for post-procedure off-line analysis.

The flow in the brachial artery was calculated as

*Baseline Flow* =  $\pi d_1^2/4 \cdot HR_1 \cdot (VTI_{S1} + VTI_{D1})$ ; where  $d_1$  is brachial artery diameter,  $HR_1$  is heart rate,  $VTI_{S1}$  is systolic VTI and  $VTI_{D1}$  is diastolic VTI at baseline.

*Reactive Hyperemia Flow* =  $\pi d_2^2/4 \cdot HR_2 \cdot (VTI_{S2} + VTI_{D2})$ ; where  $d_2$  is brachial artery diameter,  $HR_2$  is heart rate,  $VTI_{S2}$  is systolic VTI and  $VTI_{D2}$  is diastolic VTI measured immediately after release of cuff.

Percentage increase in brachial artery flow was calculated as -

*% Reactive hyperemia* =  $(\text{Reactive hyperemia flow} - \text{Baseline flow}) \times 100 / \text{Baseline flow}$

FMD was calculated as -

*FMD* =  $(d_3 - d_1) \times 100 / d_1$ ; where  $d_3$  is brachial artery diameter at 1 minute of cuff release.

### Statistical Analysis

The statistical analysis was done of SPSS 10.0 for windows. All values were expressed as mean ( standard deviation). The demographic data and risk factors were compared between the groups using chi-square and Student's t test wherever appropriate. Unpaired Student's t test was used to assess the presence of any difference in measured parameters in the four groups. A p value of  $<0.05$  was considered as statistically significant.

## RESULTS

The clinical characteristics of the individuals in the four groups are as shown in Table 1. As is evident, subjects in all the four groups were age and sex matched. There was no significant difference in the incidence of conventional cardiovascular risk factors in any of the groups. However,

**Table 1: Clinical Characteristics of the Study Population**

	Group 1	Group 2	Group 3	Group 4
N	67	38	58	35
Age*	47.58 $\pm$ 8.6	49.53 $\pm$ 8.72	48.38 $\pm$ 10.94	51.83 $\pm$ 9.19
Male sex*	58 (86.6%)	34 (89.5%)	53 (91.4%)	30 (85.7%)
Hypertension*	33 (49.3%)	24 (63.2%)	27 (46.6%)	23 (65.7%)
Dyslipidemia*	32 (47.8%)	25 (65.8%)	31 (53.4%)	18 (51.4%)
Family history*	36 (53.7%)	14 (36.8%)	25 (43.1%)	14 (40.0%)
Smoking*	15 (22.4%)	14 (36.8%)	15 (25.9%)	8 (22.9%)

\*All the p values for comparison between the groups  $> 0.05$

as was the study design, there was no diabetic in Group 1 and 3 whereas all the subjects in Group 2 and 4 had diabetes. Hence, the mean fasting and post-prandial blood glucose values (not shown) were higher in Groups 2 and 4 compared to those in Groups 1 and 3.

There was no significant difference in the brachial artery diameter and calculated brachial artery flow at baseline in the four groups (Table 2). During reactive hyperemia phase, similar degrees of increase in brachial artery flow were achieved in all the four groups. With the same degree of increase in brachial artery flow, significantly more marked flow mediated vasodilatation occurred at 1 minute in Group 1 ( $7.03 \pm 2.87\%$ ) compared to the other three groups. FMD in Group 2 ( $5.51 \pm 2.12\%$ ) was similar to that in Group 3 ( $4.56 \pm 2.70\%$ ; p value 0.195) but significantly higher than that in Group 4 ( $4.26 \pm 1.93\%$ ; p value 0.038). There was no statistically significant difference in mean FMD in Group 3 and Group 4 (p value 0.65) (Fig. 1).

## DISCUSSION

Diabetes is associated with significantly increased rates of all forms of atherosclerotic disease including coronary

artery disease.<sup>14,15,16</sup> Multiple factors such as insulin resistance, metabolic abnormalities (e.g. hyperglycemia, dyslipidemia, elevated free fatty acid levels etc.), formation of advanced glycosylation end-products, hypertension, obesity, inflammation, all individually as well as interdependently contribute to early onset and accelerated progression of atherosclerosis in diabetes.<sup>17</sup> The exact mechanisms involved in this are not entirely clear, however, endothelial dysfunction seems to play a key role. Many of the factors mentioned above can directly or indirectly result in endothelial dysfunction. Insulin itself has a direct vasodilatory effect mediated through nitric oxide,<sup>18</sup> an effect that has been shown to be impaired in patients with DM.<sup>19</sup>

Compelling data now exists to suggest that endothelial dysfunction occurs in both type<sup>19,11</sup> and type 2<sup>12</sup> diabetes as well as in subjects with insulin resistance without diabetes.<sup>20</sup> Clarkson *et al* in their study showed FMD to be significantly impaired in diabetics as compared to controls ( $5.0 \pm 3.7\%$  vs.  $9.3 \pm 3.8\%$ ; p <0.001) and the degree of impairment was directly related to the duration of diabetes.<sup>9</sup> Similarly Yu HI *et al* also found FMD to be significantly impaired in diabetics as compared to controls.<sup>12</sup> The impairment was more marked

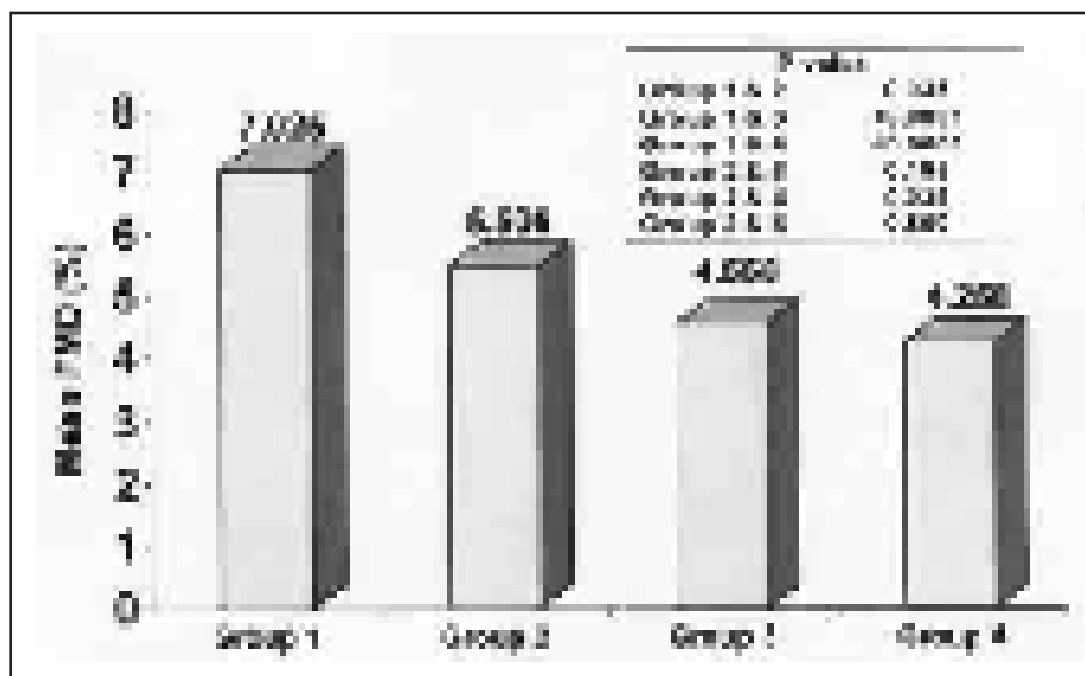


Fig 1 : Mean FMD in study groups

Table 2 : Comparison of measured parameters of FMD assessment in the study groups

	Group 1	Group 2	Group 3	Group 4
Baseline diameter (mm)*	3.853 ± 0.459	3.733 ± 0.729	3.800 ± 0.434	3.797 ± 0.670
Baseline flow (ml/min)*	129.2 ± 81.2	131.3 ± 71.5	130.5 ± 90.6	108.9 ± 47.5
Reactive hyperemia flow (ml/min)*	522.3 ± 283.2	457.6 ± 175.2	437.2 ± 234.0	399.9 ± 212.9
% hyperemia*	326.3 ± 198.8	294.7 ± 165.1	293.0 ± 218.5	282.9 ± 169.9
FMD (%)**	7.025 ± 2.87	5.506 ± 2.12	4.558 ± 2.70	4.258 ± 1.93

FMD - Flow mediated vasodilation; \*All the p values for comparison between the groups > 0.05; \*\*p values for FMD in Group 1 and 2 - 0.035; Group 1 and 3 - < 0.0001; Group 1 and 4 - < 0.0001; Group 2 and 3 - 0.195; Group 2 and 4 - 0.038; Group 3 and 4 - 0.650

in those diabetics who had associated peripheral arterial disease. Dogra *et al* showed FMD to be impaired in diabetics even in presence of near-normoglycemia and normoalbuminuria.<sup>11</sup> As was in the study by Yu HI *et al*, here again FMD impairment was more marked if diabetic complication was also present (microalbuminuria in this case). In the present study, we have also observed significant impairment of FMD in diabetics as compared to controls. Our data, thus, is in agreement with the results of the previous studies.

In addition, we found similar degree of FMD impairment in diabetics without CAD and CAD patients not having diabetes. Though, as per our knowledge, no study has attempted similar comparison, several authors have shown equally increased cardiovascular morbidity and mortality in diabetics without CAD and non-diabetics with CAD.<sup>14</sup> Haffner *et al* in their follow up study found seven year incidence of cardiovascular mortality to be 20.2% in diabetics without prior myocardial infarction and 18.8% in non-diabetics with prior myocardial infarction (p value >0.4).<sup>14</sup> Since, endothelial dysfunction has already been shown to be a good predictor of future risk of cardiovascular events, our study, by demonstrating equal impairment of FMD in patients with DM alone and in patients with CAD alone, indicates this to be the likely mechanism underlying equally increased risk of cardiovascular morbidity and mortality in these two groups. However, large population-based studies are needed to confirm these findings. Additionally, whether varying impairment in FMD in patients with DM will provide incremental value in risk stratification for future cardiovascular events need to be evaluated in large prospective studies.

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