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# Acute Effects of Blood Sugar Regulation on Endothelial Functions in Patients with Diabetes

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## Abstract

**Objectives:** Coronary artery disease (CAD) due to atherosclerosis is an important and common cause of mortality and morbidity in patients with diabetes mellitus (DM) and prediabetes. Endothelial dysfunction is a possible reason for the increased atherosclerosis in patients with diabetes. Previous studies have shown that hyperglycemia deteriorates endothelial functions, but there are not many studies have been conducted to investigate the acute effect of blood sugar regulation on impaired endothelial functions in patients with diabetes. So in this study, we evaluated the acute effect of blood sugar regulation with insulin infusion on endothelial functions in patients with diabetes.

**Materials and Methods:** Forty-four patients with diabetes who were planned to start insulin infusion and 20 healthy individuals of similar age and gender were included in this study. Flow-mediated dilatation (FMD) method was used to evaluate endothelial functions. FMD values before and after insulin infusion in patients with diabetes were calculated and compared with those in the patient population and the healthy control group.

**Results:** The mean age of the patients was 54.9±13.6 years and 54.5% were female. When the hyperglycemic and the normoglycemic periods were compared among themselves, FMD measured in the normoglycemic period was found to be significantly higher (6.13±3.11 vs 10.89±3.65, $p<0.001$). The FMD value in the group with diabetes was found to be significantly lower than that in the control group, even after treatment (10.89±3.65 vs 12.84±1.86, $p<0.006$).

**Conclusion:** In type 2 patients with DM, endothelial functions are impaired during periods of hyperglycemia. Although endothelial functions improved in the acute period after blood sugar regulation, endothelial functions still continue to be

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impaired compared with the healthy control group. Patients with diabetes may be at higher risk of vascular events during hyperglycemic periods than in normoglycemic periods.

**Keywords:** Diabetes, hyperglycemia, endothelial dysfunction, flow-mediated dilation

## Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases[1]. Coronary artery disease (CAD) due to atherosclerosis is an important and common cause of mortality and morbidity in patients with DM and prediabetic. It is considered that increased atherosclerosis in patients with diabetes develops on the basis of endothelial dysfunction[2-5]. Oxidative stress caused by hyperglycemia, increased free oxygen radicals, decreased nitric oxide (NO) synthesis, increased endothelin 1 production and hyperglycemia-related metabolic products (3-deoxyglucosone, amadori products, methylglyoxal) are important causes of endothelial dysfunction in patients with DM[6-9].

Flow-mediated vasodilation (FMD) test is a widely used non-invasive method for evaluating endothelial functions. During this test syfingomanometer cuff is inflated on the forearm for 5 min to stop arterial flow and cause ischemia. After the deployment of the cuff, hyperemia and increased shear stress will trigger the healthy endothelium to release vasodilator factors, especially NO. Basal and after ischemia brachial arter diameters are measured using ultrasound probe and an increase in the brachial arter diameter more than 10% is expected in people with healthy endothelial[10,11]. It has been shown that acute hyperglycemia with intravenous glucose in healthy individuals leads to deterioration in endothelial functions. Endothelial function is impaired in the chronic period in patients with diabetes[12-16]. However, comparison of endothelial functions between hyperglycemic and normoglycemic periods in patients with DM has not been investigated before.

This study is designed to test the hypothesis that acute worsening of endothelial functions may occur in acute hyperglycemic periods in patients with DM by comparing endothelial functions before and after insulin infusion therapy.

## Materials and Methods

Between September 2018 and October 2019, patients with type 2 DM who were planned to start an intravenous (i.v.) insulin infusion by an endocrinologist at the study center were evaluated 44 patients aged between 18 and 75 years, who signed the informed consent, were included in the study. Patients with pregnancy, ketoacidosis, malignancy and had low left ventricular ejection fraction (LVEF <40%) were excluded from the study. In the hyperglycemic period, echocardiography and FMD evaluation was performed before insulin infusion then control FMD evaluation was performed immediately after blood glucose levels were taken under control (blood glucose <180 mg/dL) and insulin infusion was discontinued. The medical history of the patients, routine laboratory tests and the other medications were recorded.

The control group was formed by 20 volunteers of similar age and gender to the patient group, who signed the informed consent, and did not have any known history of cardiovascular (CV) disease.

### Flow-mediated Vasodilation

For the brachial ultrasonographic evaluation, a 4.5-12 MHz Linear ultrasound probe (Vivid E9 4D Cardiovascular ultrasound system, Model 11L-D GE Healthcare, Chicago, USA) was used in the high resolution mode (>10 Mhz), and the brachial artery was imaged longitudinally from 5-10 cm proximal to the antecubital pit. After measuring the basal diameter of the brachial artery, the sphygmomanometer cuff was inflated to 220 mmHg,

then the cuff was deflated 5 min later. After occlusion, the measurement was made 1 min after the cuff air was released and at the end of diastole. FMD was obtained by calculating the ratio of arterial diameter change to basal artery diameter as a percentage (%).

### Statistical Analysis

The normal distribution of the variables was determined by the Kolmogorov-Smirnov test. Normally distributed continuous variables were expressed as mean (mean) and standard deviation, while non-normally distributed continuous variables were expressed as median and minimum-maximum, while categorical variables were expressed as percentage and number of cases. Continuous variables from the hyperglycemic and normoglycemic period measurements before insulin infusion were compared with the paired t-test used in the evaluation of repetitive measurements, and categorical variables were compared with the McNemar test. In comparing the control group and the DM patient group, t-test or Mann-Whitney U test was used according to the normal distribution pattern for continuous variables, while chi-square or Fisher's Exact test was used for categorical variables. A p-value of <0.05 was considered statistically significant. Ege University Faculty of Medicine Clinical Research Ethics Committee approval was obtained for the study (decision no: 18-6/43). All comparisons were made using SPSS (Statistical Package for Social Sciences) v25 (IBM, Armonk, NY, USA) statistical package program.

## Results

Forty-four patients who met the inclusion criteria and 20 healthy volunteers of similar age and gender were included in the study. The mean age of the patient group was 54.9±13.6 years and 54.5% were female. All the patients included in the study were diagnosed with type 2 DM. 63.6% of the patients had hypertension, 38.6% hyperlipidemia, 13.6% chronic renal failure, 13.6% CAD and 6.8% cerebrovascular disease. None of the patients had been diagnosed with heart failure and the mean LVEF was 60.8%±4.5% (Table 1). Left ventricular diastolic dysfunction was present in 82% of the patients. When insulin infusion was started, the blood glucose level of all patients was above 300 mg/dL and the mean was 384±76 mg/dL. While the mean HbA1c value of the patients was 11.04±2.31%, the duration of DM including the time from the first diagnosis to the study was 12.6±9.4 years. FMD values were found as 6.13%±3.11 in the first measurement. The patients received insulin infusions for 3 days. After the blood glucose level decreased below 180 mg/dL, insulin infusion was stopped and FMD measurement was repeated in normoglycemic period. The mean blood glucose at the time of control FMD measurement was 151±27 mg/dL. The mean FMD value after glycemic control in patients was calculated as 10.89±3.65%, and this value was found to be significantly lower than the hyperglycemic period (p<0.001) (Table 2, Figure 1).

The mean age of the healthy volunteers taken as the control group was similar to that of the patient group (52.6±8.6 vs 54.9±13.6 years, p=0.52). Since it has been reported that there may be changes in FMD according to gender, the female gender distribution was also found to be similar (54.5% vs 50%, p=0.736). While no additional CV disease was found in the control group, LVEF values were similar to the DM group (62.3%±3.0 vs 60.9±4.5%,

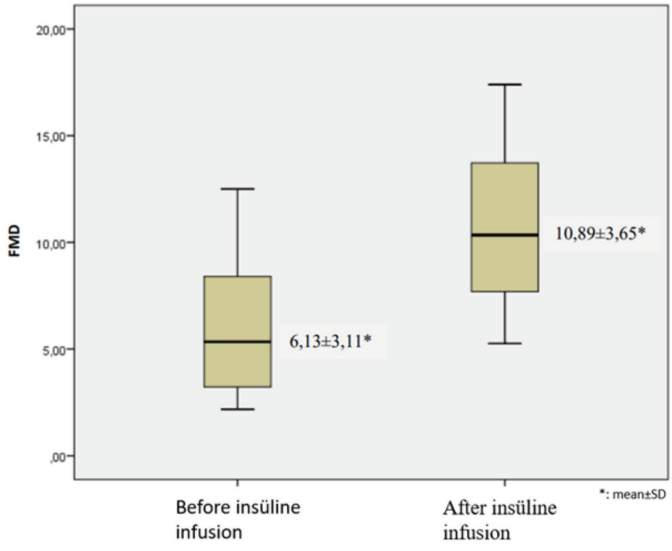


**Figure 1.** Flow mediated diameter change before and after insulin infusion

p=0.376). On the resting electrocardiography (ECG), the heart rate was higher in the patient group (83.7±10.3 vs 73.2±5.04, p=0.001). FMD measurement in the healthy group was 12.84%±1.86%. Both hyperglycemic and normoglycemic measurements obtained in the patient group were found to be significantly lower than those in the control group (p=0.001 and p=0.006) (Table 2, Figure 2).

## Discussion

The current study shows that the FMD value in the hyperglycemic period in patients with DM is significantly lower than that the healthy control group, and a significant improvement was achieved immediately after blood glucose-level regulation, however normoglisemic FMD values were still significantly lower than healthy volunteers. Decreased endothelium-dependent vascular

**Table 1.** Comparison of the main characteristics of the patient-control group

| | Patient group (n=44) | Control group (n=20) | p-value |
|---|---|---|---|
| Age (year) (mean ± SD) | 54.9±13.6 | 52.6±8.6 | 0.52 |
| Female gender [% (n)] | 54.5 (24) | 50 (10) | 0.736 |
| BMI (kg/m$^2$) (mean ± SD) | 30.5±6.25 | 26.4±3.3 | 0.001* |
| Heart rate (beat/minute) (mean ± SD) | 83.7±10.3 | 73.2±5.04 | 0.001* |
| NYHA I [% (n)] | 59.1 (26) | 100 (20) | 0.001* |
| NYHA II [% (n)] | 40.9 (18) | 0 (0) | |
| LVEF (%) (mean ± SD) | 60.9±4.5 | 62.3±3 | 0.376 |
| Hypertension [% (n)] | 63.6 (28) | 0 | |
| Hyperlipidemia [% (n)] | 38.6 (17) | 0 | |
| CAD [% (n)] | 13.6 (6) | 0 | |
| CRF [% (n)] | 13.6 (6) | 0 | |
| CVE [% (n)] | 6.8 (3) | 0 | |
| HbA1c (%) (mean ± SD) | 11.04±2.31 | NA | |
| Smoking [% (n)] | 79.5 (35) | 55 (11) | 0.043* |
| Insulin usage [% (n)] | 61.4 (27) | NA | |
| ACEI/ARB [% (n)] | 54.5 (24) | | |
| OAD [% (n)] | 52.3 (23) | NA | |
| Beta Blocker [% (n)] | 34.1 (15) | | |
| Statin [% (n)] | 29.5 (13) | | |
| ASA [% (n)] | 25 (11) | | |
| CCB [% (n)] | 9.1 (4) | | |
| Nitrate [% (n)] | 6.8 (3) | | |

*BMI: Body mass index, CAD: Coronary artery disease, CRF: Chronic renal failure, CVE: Cerebrovascular event, HbA1c: Hemoglobin A1c*

**Table 2.** FMD comparison (before infusion, after infusion, control group)

| | Before insulin infusion | After insulin infusion | Control group | p1 | p2 | p3 |
|---|---|---|---|---|---|---|
| Brachial artery basal diameter (mm) (mean ± SD) | 34.86±5.43 | 34.70±5.74 | 36.25±3.93 | 0.57 | 0.08 | 0.07 |
| Brachial artery diameter after hyperemia (mm) (mean ± SD) | 36.98±5.71 | 38.41±6.02 | 40.9±4.44 | 0.001* | 0.15 | 0.13 |
| FMD (%) (mean ± SD) | 6.13±3.11 | 10.89±3.65 | 12.84±1.86 | 0.001* | 0.001* | 0.006* |

*p1: Before and after insulin infusion, p2: Before insulin infusion and control group, p3: After insulin infusion and control group, SD: Standard deviation, FMD: Flow mediated vasodilation*

response in patients with type 2 DM is one of the earliest markers of atherosclerosis[17-19]. It has been shown that FMD is an independent predictor of CV events in patients with stable CAD, heart failure, and peripheral artery disease. Additionally, FMD was correlated with the Framingham risk score in asymptomatic individuals who underwent community screening[20-22]. Hyperglycemia in patients with diabetes could cause endothelial dysfunction by causing increased secretion of biochemicals such as AGEs, ROS, RNS, 3-deoxyglucosone, amadori products, diacylglycerol and methylglyoxal[23-26]. Tan et al.[27] evaluated FMD in 170 type 2 patients with DM and 86 healthy control groups and found that FMD was worse in patients with DM than in the healthy control and these results were consistent with AGE levels. They also showed that the fasting blood glucose values of the patient group were higher than those of the healthy group as expected, but this and many similar studies did not consider the blood glucose levels of the patients during FMD measurement. On the other hand current study showed that FMD is impaired in patients with diabetes, regardless of their glycemic status however blood glucose levels during FMD measurement affected the results and endothelial functions were worse in the hyperglycemic period than in the normoglycemic periods.

Previous studies with a small patient population shown that acute hyperglycemia causes endothelial dysfunction in healthy and diabetic persons[28-31]. Kawano et al.[32] showed a decrease in FMD in 17 normal glucose tolerance (NGT), 24 impaired glucose tolerance (IGT), 17 patients with DM at the 1st hour after the oral glucose tolerance test (OGTT). FMD in the group with NGT and IGT was increased at the 2nd hour after OGTT. However, FMD was still decreased in the DM group[32]. In contrast to the acute glycemic stress created in the OGTT, the current study evaluates the effect of relatively acute (within 3 days) regulation of chronic hyperglycemia in patients with diabetes on endothelial functions in order to more accurately represent real life. In parallel to Kawano et al.'s[32] study, significant changes were observed in FMD values in the normo- and hyperglycemic periods in this study.

Insulin hormone is secreted in response to glucose and macronutrient intake and suppresses ROS production and activation of inflammatory mechanisms. Thus, insulin is thought to have anti-inflammatory effects, while glucose has pro-inflammatory. Insulin induces endothelium-derived NO-mediated vasorelaxation in isolated rat skeletal muscle arteries. Insulin has a stimulating effect on basal blood flow[33]. It is difficult to evaluate whether the improvement in endothelial functions between the hyper- and normoglycemic periods is solely due to glycemic control or the insulin infusion in the current study. However, in a study with Goto-Kakizaki mice

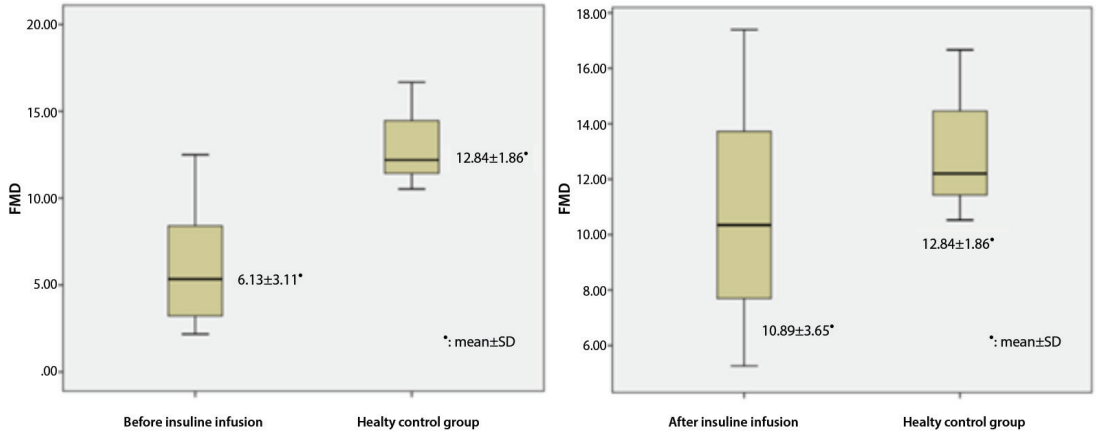


**Figure 2.** Flow-mediated dilatation of the healthy control group and patient group before and after insulin infusion
*FMD: Flow-mediated vasodilation, SD: Standard deviation*

(non-obese diabetic mouse model), monocyte adhesion was increased, which is an early step of atherosclerosis, in the aortic endothelium after glucose infusion, while after the administration of octreotide (which inhibits insulin secretion) adhesion response did not change[34,35]. This suggests that insulin levels are unimportant in the increased endothelial monocyte adhesion during hyperglisemia. According to those studies, changes in the endothelial functions in this study should be due to the control of hyperglisemia not the insulin infusion alone.

In the hyperglycemic period, endothelial dysfunction and increased atherosclerosis markers such as impaired FMD as shown in the current study or increased monocyte adhesion shown in other studies suggest that hyperglycemia may be associated with poor outcomes. In an observational clinical study, an inverse relationship was found between LVEF values measured at hospital discharge and glucose levels at admission in 500 patients who had first anterior myocardial infarction and underwent reperfusion therapy, and this results continued even after correction was performed for HbA1c levels. Such studies support the hypothesis that hyperglycemia should not only be the only risk marker but also it should be a risk factor associated with poor outcomes[36].

The results of this study support the hypothesis that hyperglycemia impairs endothelial functions, in line with other studies in the literature. However, the present study was also unique for showing that acute control of glucose levels could improve decreased endothelial functions.

### Study Limitations

The main limitation of the study was the lack of long-term follow-up of the patients in terms of clinical outcomes. Another limitation was the small patient population. The duration of diabetes of the patients is not homogeneous, although it should not have a significant effect on results because two different conditions (normo and hyperglisemic conditions) of every patient were compared between each other.

## Conclusion

In type 2 patients with DM, endothelial functions are worse during hyperglycemia compared to the normoglycemic period. Endothelial functions are worse in both the normo- and hyperglycemic periods in patients with diabetes compared in the healthy control group. This suggests that patients with diabetes should be at additional CV risk during acute hyperglycemic periods.

### Ethics

**Ethics Committee Approval:** Ege University Faculty of Medicine Clinical Research Ethics Committee approval was obtained for the study (decision no: 18-6/43).

**Informed Consent:** Written informed consent was obtained.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Mammadov G, Şimşek E, Yıldırım Şimşir I, Concept: Şimşek E, Soydaş Çınar C, Design: Mammadov G, Yıldırım Şimşir I, Data Collection and/or Processing: Mammadov G, Yıldırım Şimşir I, Analysis of Interpretation: Şimşek E, Yağmur B, Literature Search: Mammadov G, Soydaş Çınar C, Writing: Mammadov G.

**Conflict of Interest:** No conflict of interest was declared by the author.

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