Postprandial Myocardial Perfusion in Healthy Subjects and in Type 2 Diabetic Patients

Roldano Scognamiglio, MD; Christian Negut, MD; Saula Vigili De Kreutzenberg, MD; Antonio Tiengo, MD; Angelo Avogaro, MD

Background—In diabetic patients, postprandial hyperglycemia is a more powerful risk factor for cardiovascular disease than fasting hyperglycemia itself. A negative influence of acute hyperglycemia on systemic endothelial function (brachial artery) has been shown. However, myocardial perfusion during postprandial hyperglycemia has not been investigated.

Methods and Results—We evaluated the effects of a standardized mixed meal on myocardial perfusion in 20 healthy subjects and 20 consecutive patients with type 2 diabetes mellitus without macrovascular or microvascular complications. Myocardial perfusion was assessed in fasting and postprandial states by myocardial contrast echocardiography. Fasting myocardial flow velocity (β, 0.65±0.27 versus 0.67±0.24; P=NS), myocardial blood volume (MBV; 8.3±1.2 versus 8.4±2; P=NS), and myocardial blood flow (5.4±1.5 versus 5.6±2; P=NS) did not differ between control subjects and diabetic patients. In the postprandial state, β (0.67±0.24 versus 0.92±0.35; P<0.01), MBV (8.4±2 versus 10.9±2.7; P<0.01), and myocardial blood flow (5.6±2 versus 9.9±2.8; P<0.01) increased significantly in control subjects. In diabetic patients, β increased (0.65±0.27 versus 0.8±0.24; P<0.01) but MBV (8.3±1.2 versus 4.3±1.3; P<0.01) and myocardial blood flow (5.4±1.5 versus 3.4±0.9; P<0.01) decreased significantly. Changes in MBV (expressed as \[\frac{(MBV_{postprandial} - MBV_{fasting})/MBV_{fasting} \times 100}\]) were significantly correlated with postprandial glycemia levels in diabetic patients.

Conclusions—Postprandial hyperglycemia determines myocardial perfusion defects in type 2 diabetic patients. They are secondary to deterioration in microvascular function causing a decrease in MBV. In diabetic patients without microvascular or macrovascular complications, postprandial myocardial perfusion defects may represent an early marker of the atherogenic process in the coronary circulation; hence, its reversal constitutes a potential goal of treatment.

(Circulation. 2005;112:179-184.)

Key Words: coronary disease ■ diabetes mellitus ■ microcirculation ■ perfusion

Diabetes is associated with a markedly increased risk for cardiovascular disease. Abnormalities of the postprandial state, specifically postprandial hyperglycemia, have been proposed as independent risk factors for cardiovascular disease. Several studies in diabetic patients support the conclusion that postprandial hyperglycemia is a more powerful risk factor for cardiovascular disease than fasting hyperglycemia itself. It has been shown that an oxidative mechanism mediates endothelial activation induced by postprandial hyperlipidemia and hyperglycemia. This negative influence on endothelial function has been reported in both normal and diabetic subjects.

Endothelial dysfunction parallels coronary risk factors and is potentially reversible; this reversibility makes its early identification a clinically important end point. Myocardial contrast echocardiography (MCE) with microbubbles may offer an approach for noninvasive assessment of myocardial microcirculation with clinical ultrasound imaging techniques. Thus, the aim of the present study was to compare the effects of a mixed meal on myocardial perfusion, assessed by MCE, in healthy subjects and in patients with type 2 diabetes mellitus without macrovascular or microvascular complications. We hypothesized that postprandial metabolic changes (hyperglycemia and hyperlipidemia) may cause abnormalities in myocardial perfusion and that microvascular dysfunction may have a role in determining myocardial perfusion defects in type 2 diabetic patients. This is of clinical relevance because these abnormalities may represent an early marker of the atherogenic process in the coronary circulation and may constitute important targets for the treatment of the disease.

Methods

Study Populations

This prospective study involved 46 patients with diet-treated diabetes mellitus consecutively referred for follow-up evaluation. Because the aim of the study was to analyze the relationship between myocardial perfusion defects and metabolic changes induced by the postprandial state, it was necessary to select diabetic patients without factors...
influencing myocardial perfusion per se. Thus, according to the exclusion criteria, 20 patients were selected for the study. No eligible patient declined to participate. Exclusion criteria included hepatic or renal disease, microvascular and macrovascular diabetic complications, cigarette smoking, arterial hypertension, hypercholesterolemia, and hypertriglyceridemia.

The presence of retinopathy was excluded both by a detailed and comprehensive eye examination and by the acquisition of high-quality stereoscopic photographs assessed by an ophthalmologist. Nephropathy was excluded by measurement of urinary albumin excretion obtained in a timed overnight collection; microalbuminuria was defined as urinary albumin excretion greater than 30 mg/mL in at least 3 successive measurements in the absence of other factors capable of causing proteinuria (urinary infection, glomerulonephritis, kidney stone, bladder cancer, etc). Moreover, to exclude the presence of macrovascular disease, each subject was carefully characterized in terms of regional manifestations of atherosclerosis. A complete lifestyle questionnaire to obtain medical histories, parental history of cardiovascular disease, and information on smoking habits and physical activity was filled out by the patients and individually checked. Peripheral vascular disease was ruled out by ankle-brachial pressure indexes. A Doppler ultrasound of the carotid arteries was obtained in each patient to exclude carotid atherosclerotic involvement.

Diabetic patients were not taking any drugs. All patients were instructed not to change their usual dietary habits for the duration of the study. Before a patient was included in this study, he or she underwent stress perfusion imaging with technetium during exercise and dipyridamole echocardiography. Negativity of these diagnostic procedures indicated the absence of significant coronary artery disease. Twenty healthy subjects were recruited. They were matched for age, gender, and body mass index (Table 1); were nonsmokers; and had no evidence of present or past hypertension, hyperlipidemia, diabetes, coronary artery disease, or any systemic conditions. Moreover, they were not relatives of patients with diabetes, hypertension, coronary artery disease, or hyperlipidemia. All control subjects were following ad libitum diets, had no recent change in body weight, and were not taking medications. All diabetic patients and control subjects were Italian whites. The study protocol was approved by the ethics committee of the University of Padua. Healthy subjects and diabetic patients gave informed consent before being tested. The clinical characteristics of the study populations are reported in Table 1.

### Study Design

Diabetic patients and healthy subjects were studied after 12 hours of overnight fasting and 2 hours after a mixed standardized meal (Novasource Novartis; 460 kcal; 54% carbohydrate, 31% lipid, and 25% protein). Fasting blood was drawn to determine glucose, HbA1c, free fatty acids (FFA), insulin, and lipid levels (total, HDL, and LDL cholesterol and triglycerides). Fasting myocardial perfusion by contrast echocardiography was then performed. After this evaluation, control subjects and diabetic patients ate the meal in a random order (following the alphabetical order of the last name) assigned by a person not involved in study management. The meals were consumed under supervision of a nurse. Plasma levels of metabolic parameters were repeated 1 and 2 hours after eating. Myocardial perfusion was repeated 2 hours after eating.

### Echocardiographic Analysis

Echocardiograms were coded and read by 2 independent observers blinded to patient identity and the order of the studies. Regional perfusion was assessed with the 16-segment left ventricular (LV) model (1 = normal, 2 = hypokinesia, 3 = akinesia, 4 = dyskinesia). Agreement of interobserver analysis was seen in 98% of the segments visualized. Discrepancies were resolved by consensus with a third observer. LV volumes were calculated by an ellipsoid biplane area-length method. Ejection fraction (EF) was derived as EF = (EDV - ESV)/EDV, where EDV and ESV are the end-diastolic and end-systolic volumes.

### MCE Studies

MCE studies were performed in apical 4- and 2-chamber views using intermittent harmonic imaging with a phased-array system (Sonos 5500) interfaced to an S3 transducer that transmits ultrasound at a mean frequency of 1.6 MHz and receives it at 3.2 MHz. The transmit power was set at maximum, and compression was set at 50 dB. Mechanical index was 1.4. Gain settings were optimized at the beginning of each study and subsequently held constant. Continuous venous infusion of a contrast agent (Levovist, Schering AG) was performed with an infusion pump (Medrad Pulsar). An intensity-dose curve from the LV cavity was plotted to obtain the dose at which the relation was linear. This dose was used in the contrast echocardiographic studies. In each patient, to avoid significant changes in the concentration of contrast in LV cavity before and after the meal, only studies with similar values of peak LV cavity contrast intensity were analyzed. We accepted a range of ratios of peak contrast intensity in the fasting state to peak contrast intensity in the postprandial state from 0.9 to 1.1. Absence of any change in myocardial video intensity over 5 successive frames by visual assessment indicated the steady state. Once steady state was achieved, repeated imaging was obtained with sequential ECG triggering at end systole. The pulsing interval was gated to the ECG and progressively increased from 80 ms to 10 seconds. Up to 12 images acquired at each pulsing interval were recorded on an optical disk for quantitative analysis. Background-subtracted myocardial signal intensity was plotted over the increasing pulsing intervals and fit to an exponential function as described by Wei et al. to determine the slope of the ascending curve of myocardial contrast intensity (β), which provides a measure of myocardial flow velocity, and the myocardial plateau intensity, which correlates to capillary cross-sectional area and hence to myocardial blood volume (MBV). The product (β × MBV) represents a dimensionless index of myocardial blood flow (MBF). Digitized studies were coded and read by 2 independent observers blinded to patient identity and the order of the study. An index of mean regional myocardial perfusion was calculated by adding the values of regional MBF and dividing this value by the number of analyzed LV segments. In this study, the degree of interobserver and intrabrowser correlations for measurements of MBV (r = 0.94, r = 0.96, respectively) and β (r = 0.95, r = 0.96, respectively) was acceptable.

### Statistical Analysis

Results are expressed as mean ± SD for normally distributed variables (ejection fraction, end-diastolic and end-systolic volume indexes, myocardial perfusion indexes, age, blood pressure, heart rate, blood chemical variables). Comparisons between fasting and postprandial values (metabolic and hemodynamic parameters, MCE parameters, wall motion score index) within a group were made with the paired t test; comparisons between control subjects and diabetic patients were made with the t test for independent variables. Multiple comparisons to examine the change in parameters of MCE (β, MBV, cholesterol, and triglycerides).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the Study Population</th>
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<tbody>
<tr>
<td><strong>Healthy Subjects</strong></td>
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<td><strong>Diabetic Patients</strong></td>
</tr>
<tr>
<td>Age, y</td>
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<tr>
<td>Gender, M/F</td>
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<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Glucose, mg/dL</td>
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<td>HDL cholesterol, mg/dL</td>
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<td>LDL cholesterol, mg/dL</td>
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<td>Heart rate, bpm</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
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</tbody>
</table>

BMI indicates body mass index. Data are presented as mean ± SD.

*P < 0.01 vs nondiabetic controls.
MBV) from the fasting to postprandial state were performed with repeated-measures ANOVA, followed by Fisher’s protected least-significant-difference test. Correlations between MCE parameters and postprandial metabolic changes were determined by a linear least-squares method. The relationship between fasting metabolic (HbA1c, plasma glucose levels, total cholesterol, HDL and LDL, cholesterol, triglycerides, FFA, insulin) and hemodynamic (systolic and diastolic blood pressures, heart rate, rate-pressure product) variables and the occurrence of postprandial myocardial perfusion abnormalities (decrease in β, MBV, and MBF from fasting to postprandial state) was analyzed using the binary logistic regression.

Frequencies of a reduced MBV or MBF in asynergic myocardial segments were compared with the χ² test. For all statistical analyses, we used SPSS version 10.1 for Windows (SPSS Inc). A value of $P<0.05$ by the 2-tailed test was considered statistically significant.

Results

Metabolic and Hemodynamic Parameters

Type 2 diabetic patients were in good metabolic control as proved by HbA1c levels. Fasting plasma concentrations of glucose, HbA1c, total cholesterol, and LDL cholesterol were significantly higher in diabetic patients than control subjects, whereas plasma concentration of HDL cholesterol was lower in diabetic patients than in nondiabetic control subjects (Table 1). Figure 1 shows changes in circulating glucose, insulin, FFA, and triglyceride levels after the mixed meal in diabetic patients and control subjects. In nondiabetic subjects, no significant changes in plasma glucose and triglyceride levels occurred after the meal. In diabetic patients, mean plasma glucose rose from a basal value of 146±27 to 192.7±14.4 mg/dL ($P<0.01$) after the meal. Insulin increased by 652±101% in type 2 diabetics and by 840±65% in control subjects ($P=NS$ between groups). FFA significantly decreased from baseline values in type 2 diabetic patients, but the nadir value was not different between patients and control subjects (−58±4% versus −68±7%). Triglyceride level changed significantly from baseline value only in type 2 diabetics, in whom it increased after 120 minutes by 130±5% from baseline ($P<0.01$).

In this study population, postprandial values of heart rate (70±9 versus 68±6 bpm; $P=NS$), diastolic blood pressure (75±6 versus 74±6 mm Hg; $P=NS$), and systolic blood pressure (115±8 versus 116±9 mm Hg; $P=NS$) did not change significantly compared with values before the meal. Consequently, the rate-pressure product, a measure of myocardial oxygen consumption, did not change significantly.

Changes in Myocardial Function

Abnormal wall motion was present in both diabetic patients and control subjects before the meal. In the postprandial condition, LV wall motion abnormalities occurred only in diabetic patients; hypokinesis was detected in 81 of 320 myocardial segments, with an increase in wall motion score index to 1.25±0.2 ($P<0.01$). Frequency of a reduction in MBV ($P<0.001$) or MBF ($P<0.001$) was higher in hypokinetic myocardial segment (Table 2). In diabetic patients, postprandial LVEF was slightly reduced, but the difference was not significant (65±8 versus 62±5%; $P=NS$). In control subjects, LVEF did not change (65±6% versus 65±8%; $P=NS$).

Myocardial Perfusion

MCE studies showed that baseline β (0.65±0.27 versus 0.67±0.24; $P=NS$), MBV (8.3±1.2 versus 8.4±2; $P=NS$), and MBF (5.4±1.5 versus 5.6±2; $P=NS$) did not differ between control subjects and diabetic patients. Changes in myocardial perfusion after the mixed meal are shown in Table 2.

TABLE 2. Contingency Table for Regional Asynergies, MBV, and MBF

<table>
<thead>
<tr>
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<th>MBV</th>
<th>MBF</th>
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<tr>
<td></td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Hypokinetic</td>
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<tr>
<td>Normokinetic</td>
<td>76</td>
<td>163</td>
</tr>
</tbody>
</table>

I indicates increase; R, reduction.
Figure 2. In the postprandial state, β (0.67 ± 0.24 versus 0.92 ± 0.35; P < 0.01) and MBV (8.4 ± 2 versus 10.9 ± 2.7; P < 0.01) increased significantly from fasting values in control subjects. As a consequence, postprandial MBF also increased in nondiabetic subjects (5.6 ± 2 versus 9.9 ± 2.8; P < 0.01). In diabetic patients, in the postprandial state, β increased (0.65 ± 0.27 versus 0.8 ± 0.24; P < 0.01), whereas MBV (8.3 ± 1.2 versus 4.3 ± 1.3; P < 0.01) and MBF (5.4 ± 1.5 versus 3.4 ± 0.9; P < 0.01) decreased significantly. Figure 2 shows changes in β, MBV, and MBF in diabetic patients and control subjects. Figure 3 depicts an example of acoustic intensity–versus-time plots and actual MCE images showing a reduction in MBV after a meal in a diabetic patient.

Discussion

In this study, a significant decrease in myocardial perfusion has been shown in type 2 diabetic patients during the postprandial state. In association with perfusion defects, regional wall motion abnormalities occurred without significant changes in global LV function. The perfusion defects are caused by a decrease in MBV, which correlated with postprandial hyperglycemia. In normal subjects, no defects in myocardial perfusion have been demonstrated in the postprandial state despite the suggestion that hyperglycemia and hypertriglyceridemia also produce an endothelial dysfunction in normal subjects.9,21,22 Previous studies showed that acute hyperglycemia could cause endothelial dysfunction,23,24 and a rapid decrease in flow-mediated vasodilation in the brachial artery has been shown in the postprandial phase in type 2 diabetic patients.25 Similarly, a negative influence of postprandial hypertriglyceridemia on endothelial function has been reported in diabetic subjects.10,11 Some apparent controversies with the results of the present study can be explained by differences in methodology. In these studies, endothelial function was determined after a standardized, yet unphysiological, challenge such as the oral glucose test. Moreover, flow-mediated endothelium-dependent vasodilation of brachial artery as a surrogate marker of endothelial function was adopted. However, it has been demonstrated that peripheral flow response to transient arterial forearm occlusion does not reflect myocardial perfusion reserve.26 Thus, assessment of the systemic microcirculation cannot be used as a surrogate marker of myocardial perfusion. The lack of correlation between changes in coronary and systemic microcirculation indicates different mechanisms of microvascular activation or regulation, and extrapolations from findings in the 2 vascular beds are not suitable.26

The results of the present study showed that the negative effect of the postprandial phase specifically involves the coronary capillary circulation. An altered insulin-mediated recruitment of capillary at the heart level may contribute to this impairment in myocardial microcirculation. It has been shown that insulin rapidly recruits skeletal muscle capillaries in vivo by a nitric oxide–dependent action28 and that conditions of insulin resistance may block insulin-mediated glucose uptake and capillary recruitment.29

Our study population assumed a physiological mixed meal. At variance with the studies in which only oral glucose was used but in agreement with those in which a mixed meal was used, an increased triglyceride concentration was observed in patients with diabetes. A negative influence of postprandial hypertriglyceridemia on endothelial function has been reported in diabetic subjects.10,11 This metabolic condition, which is now recognized as an independent predictor of...
cardiovascular events,\textsuperscript{11} may have played a role in determining our results. It must also be stressed that the physiological postprandial state is characterized not only by hyperglycemia and hypertriglyceridemia but also by activation of the adrenergic nervous system.\textsuperscript{30} All these conditions are equally enhanced by type 2 diabetes.

In diabetic patients, postprandial reduction in myocardial perfusion is determined by a reduction in MBV. This index, assessed by MCE, is related significantly to microvascular indexes (total microvascular density, capillary density, capillary area) in biopsied myocardial segments, whereas the parameter $\beta$ (myocardial flow velocity) did not relate to microvascular structural findings.\textsuperscript{31} Conversely, $\beta$ reserve decreases in the myocardium subtended by coronary vessels with significant stenosis, and $\beta$ is the only parameter able to discriminate various grades of epicardial vessel stenosis.\textsuperscript{32}

Possible mechanisms to explain the increase in myocardial flow velocity but decrease in MBV in diabetic patients during the postprandial state may be derived by the recent demonstration of the role of capillaries in the regulation of coronary blood flow beyond the autoregulatory range.\textsuperscript{33} In the absence of significant coronary stenosis, hyperemic response is invariably characterized by an increase in flow velocity.\textsuperscript{32} When arteriolar tone is exhausted so that epicardial coronary blood flow becomes dependent on coronary driving pressure, MBV decreases to maintain a constant capillary hydrostatic pressure that takes precedence over myocardial oxygen delivery.\textsuperscript{33}

Although global impairment of myocardial perfusion has been demonstrated, defects were more evident in some asynergic myocardial segments. These findings are in agreement with previous anatomical studies in animal models of diabetes showing regional abnormalities in coronary microvasculature characterized by microaneurysms, tortuosity, and multiple foci of luminal narrowing characteristic of vessels in spasm.\textsuperscript{34,35} Of note, similar regional microvascular alterations were also identified in an experimental model of cardiomyopathy,\textsuperscript{36} and impairment in regional MBF reserve has been demonstrated in patients with idiopathic dilated cardiomyopathy.\textsuperscript{37}

Thus, the present data suggest that myocardial perfusion defects in the postprandial state may be related to deterioration in coronary microvascular circulation. The microcirculation is an important target of hyperglycemic damage, specifically postprandial hyperglycemia. Considerable data that have accumulated over the past 5 years indicate that elevated postprandial glucose levels increase the risk of cardiovascular disease. Information from in vitro and in vivo studies has provided biochemical mechanisms by which increases in plasma glucose levels may produce cardiovascular damage. These include activation of the polyol and glucosamine pathways, increased advanced glycation end-product synthesis, activation of protein kinase C, and increased generation of free radicals.\textsuperscript{2} Myocardial perfusion defects induced by postprandial hyperglycemia may represent an early marker of such damage and may play a role in the pathogenesis of coronary vessel obstructive disease. The
response-to-injury hypothesis of atherosclerosis states that the initial damage affects the arterial endothelium, leading to endothelial dysfunction.\textsuperscript{38}

In conclusion, acute hyperglycemia in the postprandial state is associated with myocardial perfusion defects in type 2 diabetic patients. These defects are secondary to deterioration in microvascular function, which may represent an early marker of the atherogenic process in the coronary circulation. From a clinical point of view, controlling postprandial hyperglycemia and myocardial perfusion defects may constitute important goals in the treatment of the disease.

Acknowledgment
We thank Claudio Bellini for his technical assistance.

References


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Circulation. 2005;112:179-184; originally published online July 5, 2005;
doi: 10.1161/CIRCULATIONAHA.104.495127
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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