Diabetes Mellitus as a Predictor for Radial Artery Vasoreactivity in Patients Undergoing Coronary Artery Bypass Grafting

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Objectives
Our purpose was to examine the impact of diabetes mellitus (DM) on vasoreactivity and endothelial function of radial artery (RA) grafts ex vivo.

Background
The arteriopathy associated with DM may influence the surgeon’s choice of conduits for revascularization. Arterial conduits and especially the RA are prone to vasospasm in the perioperative period.

Methods
The study population consisted of 98 patients with coronary artery disease undergoing coronary artery bypass grafting by using RA grafts. The maximum contractions of RA segments induced by K+ (66 mmol/l) and clinically important vasoconstrictors such as adrenaline (5 × 10⁻⁵ mol/l), angiotensin II (10⁻⁶ mol/l), and prostaglandin F2α (PGF2α) (10⁻⁶ mol/l) were recorded. Relaxation of RA rings to carbachol (10⁻⁴ mol/l) was used as a measure of endothelial function. Multivariate analysis was then applied to determine the role of clinical characteristics on the vasomotor responses to these agents.

Results
Vessels from patients with DM had greater contractions in response to adrenaline (p < 0.05), angiotensin (p < 0.05), and PGF2α (p < 0.01) compared with non-DM vessels, despite the similar vasoconstrictions induced by high K+ (p = NS). Diabetes mellitus was also associated with smaller vasorelaxations in response to carbachol (p < 0.001). In multivariate analysis, DM was an independent predictor of RA contractions in response to adrenaline (β [SE] 3.085 [1.410], p = 0.031), angiotensin II (β [SE] 3.838 [1.552], p = 0.015), and PGF2α (β [SE] 4.609 [1.908], p = 0.018) but not K+ (p = NS). Diabetes mellitus was also independently associated with the vasorelaxations in response to carbachol (β [SE] –15.645 [2.622], p = 0.0001).

Conclusions
Diabetes mellitus is associated with impaired endothelial function and greater contractions of RA grafts in response to all of the clinically relevant vasoconstrictors. These findings suggest that the RA of diabetic patients may be more prone to spasm in response to endogenous vasoconstrictors, an observation with important implications for surgeons’ choice of conduits in this cohort of patients. (J Am Coll Cardiol 2007;50:1047–53) © 2007 by the American College of Cardiology Foundation

Recent advances in percutaneous interventions have markedly influenced the demographic profile of patients being referred for coronary artery bypass grafting (CABG) (1). Whereas percutaneous coronary intervention has made significant inroads in the revascularization of patients with well-defined and discrete coronary lesions, patients with more diffuse coronary artery disease (CAD) still do significantly better in terms of survival and reduced need for further intervention with CABG (2,3). Coronary artery disease is common in adults with diabetes mellitus (DM) compared with nondiabetic patients and carries a worse prognosis (4). As the incidence of DM continues to rise worldwide and as CAD in diabetic patients tends to be more aggressive and diffuse in nature (5), these patients make up an ever-increasing proportion of the surgical cohort.

Diabetic patients are at increased risk of mortality and morbidity after any form of coronary revascularization (4,6), and a meticulous surgical strategy is therefore mandatory. The internal mammary artery is especially resistant to arteriosclerosis and, even in diabetic patients, has an established long-term patency and survival advantage over either saphenous vein or radial artery (RA) grafts (7,8). There is also evidence to suggest that diabetic patients do better with more arterial grafts (7,9), but this must be counterbalanced against the increased risks of sternal wound complications in these patients (10).
Although prone to vasospasm, the RA is the third most commonly used conduit in CABG (11), and reported patency data suggest that it has at least as good patency in the short-to-medium term as saphenous vein grafts and may be possibly superior in the long term (8,12). Early RA graft spasm is reported to occur in up to 10% of patients after CABG and may lead to perioperative myocardial infarction or death (11). Longer-term RA graft patency is now known to be significantly affected by the caliber of and the degree of stenosis in the native coronary vessel and the quality of distal run off (13). Furthermore, RAs from diabetic patients are more prone to calcium deposition, which may make the conduit unusable in many of these patients (14).

Diabetes mellitus is associated with endothelial dysfunction (15,16), but it is unknown whether diabetic patients are more or less prone to RA vasospasm compared with nondiabetic subjects in the perioperative period, and what effect this may have on longer-term graft patency. Using functional myography, we sought to examine whether DM has any effect on the tendency for RA vasospasm in CABG patients, by determining the vasmotor responses of RA grafts to several endogenous vasoconstrictors such as adrenaline, angiotensin II, and prostaglandin F2α (PGF2α). To further investigate the underlying mechanisms of this effect, we examined whether the potential effects of DM on the vasoconstrictive responses of RA to endogenous vasoconstrictors were due to a direct impairment of endothelial function.

Methods

Study protocol and population. The study population consisted of 98 patients undergoing elective CABG in the John Radcliffe Hospital, Oxford, United Kingdom (Table 1). Diabetes mellitus was defined in accordance with the National Data Group Criteria (17). Exclusion criteria were evidence for hepatic or hematologic abnormalities, presence of inflammatory or renal disease, as well as acute coronary event during the last 3 months before recruitment.

During the recruitment period, all patients scheduled for CABG by using RA grafts, who fulfilled the inclusion criteria and agreed to participate, were recruited for the study. To examine the effect of DM on the vasmotor responses of RA grafts, we recruited 75 nondiabetic and 23 diabetic patients (with glycosylated hemoglobin [HbA1c] 7.45 ± 0.18%). Nondiabetic subjects were matched to diabetic patients according to age, gender, and classic risk factors for atherosclerosis (Table 1); they had HbA1c <6.0%, body mass index <29.9 kg/m², and no clinical evidence of insulin resistance. The study was approved by the Oxfordshire Research Ethics Committee (OXREC number CA0129), and patients who gave informed written consent were enrolled.

Specimen collection and preparation. Radial artery conduits were harvested, as previously described (18,19), using a longitudinal forearm incision, and careful dissection of the artery and its pedicle with scissors, clips, and diathermy. Radial arteries with any evidence of macroscopic calcification were rejected and not included in the study. After harvesting, a 1- to 2-cm segment of the most distal portion of the RA was immediately placed in chilled (4°C) physiological saline solution (PSS) and transferred to the laboratory on ice where fat and periarteriolar connective tissue was removed. The vessel was divided into four 2- to 3-mm rings and mounted on steel wires in a multichamber automated myograph (Danish Myo Ltd., Winston-Salem, North Carolina). The rings were allowed to equilibrate for 60 min in aerated PSS (95% O₂, 5% CO₂) at 37°C. Throughout preparation and mounting, strenuous efforts were made to minimize any potential trauma to the vascular endothelium.

Experimental protocol. The experimental protocol has been previously described in detail (18,19). Briefly, after the equilibration and mounting process (see the preceding text), a resting tension of 3 g (approximately equivalent to 100
mm Hg arterial pressure) was gradually applied to the RA rings by slowly lengthening the distance between the mounting hooks. Once tension had been stabilized, the rings were kept at this same length throughout the experiment. The incubating PSS was then replaced with iso-osmolar high K+/H11001 PSS (containing 66 mmol/l KCl) to test viability and to produce a controlled contraction. The rings were then washed with PSS to allow relaxation to baseline before being contracted again with either high K+/H11001 PSS, PGF2α (10⁻⁶ mol/l), adrenaline (5 × 10⁻⁵ mol/l), or angiotensin II (10⁻⁶ mol/l); the concentrations of vasoconstrictors used were predetermined from preliminary experiments (Fig. 1). To avoid any adverse effects from repeated contractions and to avoid accumulation of vasoconstrictors in the chambers of the myograph, each RA ring was only contracted with a single vasoconstrictor.

Assessment of endothelial function. The degree of relaxation induced by the acetylcholine analogue carbachol (10⁻⁴ mol/l) after precontraction with PGF2α (10⁻⁶ mol/l) was recorded and used as a measure of endothelial function.

Drugs and solutions. Physiological saline solution was freshly prepared for each experiment and was composed of Na⁺ = 137 mmol/l, K⁺ = 5.9 mmol/l, Mg²⁺ = 1.2 mmol/l, Ca²⁺ = 2.5 mmol/l, Cl⁻ = 134 mmol/l, H₂PO₄⁻ = 1.2 mmol/l, HCO₃⁻ = 15.5 mmol/l, and glucose 11.5 mmol/l, pH 7.4. The following stock solutions of drugs were prepared in PSS and stored in frozen 0.8-ml aliquots: adrenaline (5 × 10⁻⁴ mol/l), angiotensin II (10⁻⁴ mol/l), PGF2α (10⁻⁴ mol/l), and carbachol (10⁻² mol/l) (supplied from Sigma-Aldrich Chemicals, Poole, United Kingdom).

Statistical analysis. The result of each experiment was logged using Maclab data recording software (ADInstruments, Bella Vista, Australia). Power calculations, based on preliminary data from our laboratory, showed that a total number of 85 patients was able to detect a 20% difference in the vasomotor responses of RA to PGF2α between nondiabetic and diabetic patients (3:1 ratio), with α = 0.05 and statistical power 80%. However, we increased the total number of patients to 98, to allow for 15% drop-outs. Data were tested for normal distribution by using the Kolmokorov-Smirnov test. Normally distributed variables are expressed as means ± standard error of the mean, and non-normally distributed variables (vasomotor responses to high K+) were log-transformed for all analyses and are presented in the nonlogarithmic format as median (25th to 75th percentile). The logs of the non-normally distributed variable had normal distribution. Comparisons between 2 groups were performed by unpaired t test as appropriate. To examine whether DM was an independent predictor of the vasomotor responses of RA to each of the agonists we used, we performed linear regression by using the maximum contractions in response to PGF2α, K+, adrenaline, or angiotensin II or the maximum relaxation in response to carbachol as dependent variables, and as independent variables DM and those of the clinical factors (gender, dyslipidemia, smoking, hypertension, body weight, and age), which showed significant correlations with the respective dependent variable in univariate analysis at 15% significance level. A backward elimination procedure was applied in all multivariate models (using p < 0.05 as the threshold for removing a variable from the model, by using the backward method in SPSS software [SPSS Inc., Chicago, Illinois]). All the analyses were performed by using SPSS software version 12.0.

Results

The demographic characteristics of the participants are presented in Table 1. The concentration of each constrictor was predetermined in a pilot study of 4 patients, using dose response curves (Fig. 1) and selected so as to produce near maximal contraction. As expected, there were significant differences.
correlations of the vasomotor responses of RA to different vasoconstrictors such as adrenaline, angiotensin II, and high K+ (r = 0.332, p = 0.001). In univariate analysis, the contractions in response to adrenaline were correlated with the contractions in response to angiotensin II (r = 0.339, p = 0.001), PGF2α (r = 0.332, p = 0.001), and high K+ (r = 0.239, p = 0.018). Similarly, the contractions in response to angiotensin II correlated with the contractions in response to PGF2α (r = 0.732, p = 0.0001) and high K+ (r = 0.427, p = 0.0001), whereas the contractions in response to PGF2α also correlated with the contractions in response to high K+ (r = 0.570, p = 0.0001).

DM and vasomotor responses of RA grafts. Radial artery grafts from patients with DM appeared to have significantly greater vasomotor responses to adrenaline, PGF2α, and angiotensin II (Fig. 2). However, there was no significant difference in the vasomotor responses of RA to high K+ between patients with DM (median [25th to 75th percentile] 13.05 g [7.78 to 21.07 g]) and patients without DM (median [25th to 75th percentile] 11.91 g [7.37 to 20.88 g], p = NS) (Fig. 3A).

Body mass index was also correlated in univariate analysis with the vasomotor responses to all the examined vasoconstrictors (adrenaline [r = 0.353, p = 0.001], PGF2α [r = 0.312, p = 0.002], angiotensin II [r = 0.366, p = 0.001], including high K+ [r = 0.290, p = 0.004]).

Carbachol (10^{-4} mol/l) induced a significantly lower vasorelaxation in PGF2α (10^{-6} mol/l) precontracted RA rings from patients with DM compared with nondiabetic patients (Fig. 3B). However, the vasorelaxations in response to carbachol were not correlated with the vasoconstrictive responses to K+ (r = 0.052, p = 0.614), adrenaline (r = -0.081, p = 0.429), angiotensin (r = -0.033, p = 0.744), or PGF2α (r = -0.038, p = 0.708), suggesting that the effect of DM on vasomotor responses to endogenous vasoconstrictors is independent of its effects on endothelial function.

Results from multivariate analysis. In multivariate analysis, the only independent predictors for the contractions in response to PGF2α were DM (β [SE] 4.609 [1.908], p = 0.018) and body weight (β [SE] 0.162 [0.060], p = 0.008). Similarly, the only independent predictors for the vasomotor responses to angiotensin II and adrenaline were DM (β [SE] 3.838 [1.552], p = 0.015, and β [SE] 3.085 [1.410], p = 0.031, respectively) and body weight (β [SE] 0.186 [0.051], p = 0.0001, and β [SE] 0.168 [0.047], p = 0.001,
respectively). For the vasomotor responses to high K+, body weight was the only independent predictor ($\beta$ [SE] 0.225 [0.063], $p = 0.001$). The only independent predictors for the vasorelaxations of RA in response to carbachol were male gender ($\beta$ [SE] 6.31 [2.62], $p = 0.018$) and DM ($\beta$ [SE] $-15.645$ [2.622], $p = 0.0001$). Interestingly, the effect of DM on the vasomotor responses to all the examined vasoconstrictors was independent from the effects on endothelial function.

**Discussion**

In the present study we have demonstrated that RAs obtained from diabetic patients with CAD have greater tendency to spasm compared with RAs from nondiabetic patients, and that RAs from patients with DM have impaired endothelial function. In multivariate analysis, only DM and body weight were independent predictors of the vasomotor responses of RAs to all endogenous vasoconstrictors. Interestingly, the effect of DM on the vasoconstrictive responses of RAs to endogenous agonists was independent from its effects on endothelial function. These findings provide new insights into the complex mechanisms of vasospasm in RA grafts, suggesting that DM may induce vasospasm in RA grafts by acting directly to the vascular wall, independently from its effects on vascular endothelium.

**Vascular endothelium and vasospasm in RAs.** Evidence suggests that substances such as angiotensin II (20), catecholamines, and PGF2α (21) are the main endogenous vasoconstrictors responsible for the spasm of RA grafts during the early postoperative period. The circulating levels of many of these substances are increased during and after cardiac surgery (22), and their direct effects on RA graft segments ex vivo are used as a reliable model system to examine the mechanisms of vasospasm in these grafts (19).

However, the propensity of these grafts toward spasm is not solely a function of the muscular wall, but endothelium-derived nitric oxide (NO), as a major vasorelaxant in human vasculature (23), may also be implicated into this phenomenon (24).

In the present study we examined whether the ex vivo vasomotor responses of RA grafts to endogenous vasoconstrictors such as angiotensin II, PGF2α, and adrenaline are correlated with the vasorelaxations induced by a NO-receptor-mediated vasorelaxant, carbachol. Surprisingly, the vasorelaxations in response to carbachol were not correlated with the vasomotor responses to any of the vasoconstrictive agents, implying that NO may not be a major modulator of the vasoconstrictive responses of RA grafts to adrenaline, PGF2α, or angiotensin II. On the contrary, a direct effect of these vasoconstrictors on the vascular wall is more likely to be responsible for their vasospastic effect on these grafts.

**DM and vasospasm in RAs.** Smooth muscle function in patients with DM is still an unresolved issue. There is compelling evidence for increased production of vasoconstrictors such as angiotensin II, endothelin 1, cyclooxygenase, and lipoxygenase products of arachidonic acid metabolism in diabetic vessels (25). These molecules as well as other cytokines and growth factors that increase vascular tone seem to contribute to the microvascular, macrovascular, and renal complications in diabetes. There is also evidence to suggest that patients with long-standing DM have impaired vasoreactivity, which may be attributable to higher levels of calcification in the vessel wall (14). The increased levels of endogenous vasoconstrictors combined with the impairment of vascular smooth muscle cells in arteries from patients with DM (23) lead to the hypothesis that the whole arterial tree in DM may be more prone to vasospasm. However, it is unknown whether RA from diabetic patients used as CABG grafts have higher incidence of spasm compared with RA from nondiabetic patients.

In the present study we have demonstrated that RA grafts from patients with DM have impaired endothelial function and are more prone to vasospasm. These findings are compatible with previous reports showing that diabetic vessels are more prone to vasoconstriction, but this is still a topic of controversy (26–28). Although extrapolation of our findings would suggest that RAs from diabetic patients may be more prone to early occlusion than RAs from nondiabetic patients, the recently reported interim results of RAPS (Radial Artery Patency Study), a randomized trial comparing radial and saphenous vein graft patency using angiographic follow-up, failed to show diabetes as an independent risk factor for RA occlusion at 1-year follow-up (12). Therefore, we hypothesize that the increased RA contractility seen in vessels from diabetic patients may lead to an increased incidence of immediate postoperative RA spasm and perioperative ischemia/infarction without directly impacting on short-to-medium-term patency.

Another finding of the present study was the association between body mass index (or body weight) and the vasospastic responses to all the agents used, including high K+. One could speculate that patients with higher body weight are more likely to have more muscular RA grafts, leading to a nonspecific association between body mass index (or body weight) and the vasoconstrictive responses. Importantly, these associations were independent of the presence of DM, as it was demonstrated in multivariate analysis. In any case, this is a finding requiring further exploration.

It is likely that the decreased NO bioavailability in vessels from patients with DM may affect vasoreactivity, because endothelium-derived NO is a major vasorelaxant in human vasculature (23). Indeed, diabetes-induced impairment of endothelial function is a systemic phenomenon (23,29) observed in the brachial artery (30), the coronaries (31), as well as in vessels used as grafts in CABG, such as internal mammary arteries (23) and saphenous veins (32). Endothelial dysfunction in DM is multifactorial in nature, including decreased NO synthesis (33) and increased NO breakdown due to the higher oxidative stress status (34,35). In particular, evidence suggests that in vessels from diabetic patients,
endothelial NO synthase is largely “uncoupled” and it is a source of superoxide radicals instead of NO (16). Further to the increased oxidative stress status, other mechanisms such as the proinflammatory stimuli or the presence of insulin resistance are also key mechanisms inducing endothelial dysfunction in diabetic patients (36).

In the present study we confirmed that DM is associated with decreased NO bioavailability in RA grafts, from patients undergoing CABG. However, we have also demonstrated that the vasoconstrictive responses of RA grafts to endogenous vasoconstrictors implicated in vasospasm, such as angiotensin II, PGF2α, and adrenaline, are independent from NO bioavailability in the same vessels, as determined by the vasorelaxations in response to carbachol. These findings suggest that the stimulatory effect of DM on RA vasospasm is more likely to be due to direct damage of the vascular wall, including impairment of vascular smooth muscle cells or vascular innervation, rather than due to the coexisting decreased NO bioavailability. Indeed, our findings are compatible with recent reports from animal models, suggesting increased aortic vasoactivity in a diabetic rat model (37), which may be due to direct effects of DM on the functional coupling of angiotensin II–angiotensin type 1 receptors (38) or the increased responsiveness of vascular smooth muscle cells to catecholamines (37).

A limitation of our study is the absence of any direct measurement of insulin resistance in the study population. Therefore, the presence of patients with insulin resistance (but without clinically documented DM) among the non-diabetic group cannot be excluded. Furthermore, ex vivo measurements of vasorelaxations of RA grafts in response to carbachol or vasomotor responses to the examined vasoconstrictors should not be directly extrapolated to in vivo conditions. Although this is considered to be a good model system to study the properties of these vessels (39) and it provides similar results to noninvasive measures of vascular function (40), any extrapolation to a clinical setting should be made with caution because the concentrations of these vasoactive agents used in this model are higher than those observed in human circulation in vivo.

**Conclusions**

This is the first study demonstrating that DM is an independent predictor for the vasomotor responses of RA grafts to physiological vasoconstrictors such as adrenaline, PGF2α, and angiotensin II. Furthermore, we provide evidence that endothelial dysfunction also observed in RA from diabetic patients is not related to the increased vasomotor responses of these grafts to endogenous vasoconstrictors. Further prospective clinical data are required to determine whether RA is a rather inferior CABG graft type in diabetic patients, as a result of its higher likelihood for perioperative spasm.

**REFERENCES**


