Eur J Vasc Endovasc Surg **14**, 41–47 (1997)

Dilatation of Saphenous Vein Grafts by Nitric Oxide*

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Objectives: *To investigate firstly whether flow-dependent vasodilation is maintained in vein grafts, and secondly whether nitric oxide donors dilate vein grafts to improve the flow through graft stenoses.*
Design, materials and methods: *The vasodilatation of mature patent vein grafts, in response to reactive hyperaemia and glyceryl trinitrate (GTN), was assessed by the change in external diameter using duplex ultrasonography. The severity (ratio of proximal systolic velocity, V1, to peak systolic velocity at the stenosis, V2, of vein graft stenoses was determined by duplex ultrasonography before and after 24 h of local application of GTN patches.*
Results: *In post-occlusion hyperaemia the diameter of patent distal vein grafts* (n=7) *increased to a maximum of* $112 \pm 1.9\%$ *of resting diameter after 2 min,* $p=0.026$. *The diameter increased further to* $117 \pm 2.5\%$ *of the resting value 5 min after oral GTN* (n=5), $p=0.007$. *The velocity ratio, V2/V1, through graft stenoses* (n=6) *decreased by* $20 \pm 5\%$ *after application of GTN patches, principally as a result of reduction in V2, mean difference 0.8,* $p=0.15$. *The changes in response to GTN were more evident for proximal than distal vein graft stenoses.*
Conclusion: *Flow-induced vasodilatation responses, which have been attributed to the endothelial release of nitric oxide, are maintained in patent vein grafts: the grafts dilate even further in response to GTN. The application of GTN patches close to a vein graft stenosis appears to reduce the velocity ratio through vein graft stenoses. GTN patches might be used to reduce the risk of graft occlusion when there is a delay between the detection and the treatment of haemodynamically significant graft stenoses.*

Key Words: Vein graft; Stenosis; Nitric oxide; Hyperaemia; Glyceryl trinitrate.

Introduction

Many changes occur in the saphenous vein graft as it adapts to the new haemodynamic environment of an arterial bypass graft. These changes include the thickening of the vessel wall and changes in diameter.[1] Most of the changes in vessel diameter occur during the first 3 months after implantation and are related to the new shear stresses imposed on the graft.[1] Many would consider that following this first period of adaptation the matured vein graft was maintained at a fixed diameter, not amenable to further regulation. Studies of excised vein grafts have provided conflicting evidence on this matter. *In vitro* studies have shown that excised aorto-coronary vein grafts maintain endothelium-dependent relaxation responses.[2] In contrast, similar studies of excised femorodistal vein grafts have indicated that mature infrainguinal vein grafts do not maintain their endothelium-dependent relaxation responses.[3] The superficial anatomy of many of these infrainguinal vein grafts suggests that regulation of vein graft diameter could be studied *in vivo*. The ultrasonographic techniques for *in vivo* studies have been described by Celermajer *et al.*[4] These authors have used ultrasonography to demonstrate the increased diameter of the femoral artery in response to hyperaemic flow.[4] This flow-dependent vasodilatation has been attributed to the increased flow stimulating the production of nitric oxide from endothelium, with the nitric oxide triggering vasorelaxation of the underlying smooth muscle. In the presence of factors which injure endothelium, hypercholesterolaemia and smoking, the flow-dependent relaxation of the femoral artery is blunted.[4]

Here, we have investigated the responses of infrainguinal vein grafts to hyperaemic flow or nitric oxide donors (e.g. glyceryl trinitrate) *in vivo*. In subsequent investigations we have attempted to exploit these responses to regulate graft diameter temporarily and improve the flow characteristics through vein graft stenoses.

* Winner of the Young Investigators Award at the 10th annual meeting of the European Society for Vascular Surgery, Venice, Italy (12–15 September 1996).
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1078–5884/97/070041+07 $12.00/0

Table 1. Hyperaemic responses in patients with patent vein grafts.

Patient	Age	Graft type	Age of graft (months)	Systolic BP (mmHg)	ABPI	Serum cholesterol (mM)	Graft d at rest (mm)	Max post-occlusion d (mm)	Peak post-occlusion d at (s)	Max d after GTN (mm)
A	74	Femoropopliteal *in situ*	9	155	1	5.3	5.1	5.9	120	6.1
B	67	Femorotibial reversed	9	170	1	6.9	6.7	7.4	120	7.6
C	69	Femoropopliteal reversed	6	162	1	6.6	7.0	7.4	120	7.7
D	72	Femorotibial *in situ*	6	140	0.9	5.9	5.2	6.1	120	6.4
E	72	Femorotibial *in situ*	9	160	0.9	5.5	6.9	7.7	90	nd
F	64	Femorotibial reversed	6	135	1	5.3	6.8	7.2	160	nd
G	57	Femorotibial reversed	6	125	1	6.1	9.5	11.2	120	11.5

All patients were male; BP = blood pressure, ABPI = Ankle brachial pressure index, d = diameter [maximum (max) diameter refers to that achieved following reactive hyperaemia or glyceryl trinitrate (GTN)], nd = not done.

Table 2. Patients with vein graft stenosis receiving GTN patches.

Patient	Age	Sex	Graft type	Site of stenosis	Age of graft (months)	Severity of stenosis
1	74	F	Femorotibial *in situ*	Proximal anastomosis	3	70%
2	82	M	Femorotibioperoneal trunk	Distal graft	3	90%
3	79	F	Femorotibial *in situ*	Proximal graft	3	70%
4	80	F	Femorotibial *in situ*	Distal graft	3	90%
5	82	M	Femoroperoneal *in situ*	Proximal graft	1	50%
6	70	M	Femorotibial *in situ*	Proximal anastomosis	3	70%

Patients and Methods

From January 1992 all patients undergoing vein bypass at Charing Cross Hospital were entered into a graft surveillance programme using colour-flow Doppler ultrasonography.[5] Regular graft surveillance was performed by an experienced vascular technologist using an Ultramark 9 HDI scanner (Advanced Technology Laboratories, Bothell, Washington). From these we selected seven patients with widely patent grafts at 6 or 9 months after implantation for *in vivo* studies of vein graft vasodilatation: the patients' characteristics are shown in Table 1. For this first study we excluded patients with diabetes, patients taking nitrates and smokers. Smoking status was monitored using whole blood carboxyhaemoglobin levels. We also selected six patients with haemodynamically significant graft stenoses (four proximal stenoses, two distal stenoses) to investigate whether flow through the stenosis could be improved by the application of glyceryl trinitrate (GTN) patches: the characteristics of these patients are shown in Table 2. Both these studies were approved by the local ethical committee.

Vasodilatation of mature vein grafts

The protocol for these investigations was adapted from that of Celemajer *et al.*[4] Patients rested supine for 15 min before investigations. The vein graft was imaged in longitudinal section in the adductor canal using a 5MHz probe. The internal and external diameters were measured, using a mean of three readings. A dual channel laser-Doppler blood flow monitor (Moor Instruments, Model MBF3/D Exeter, U.K.) was used to measure cutaneous capillary flow on the lateral aspect of the calf, 10 cm distal to the knee joint, and basal flow (cellular flux) recorded. A thigh cuff was inflated to 50 mmHg above the systolic blood pressure and the inflation maintained for 4 min, during which period flow in the vein graft and cell flux through the calf skin capillaries fell to zero. Following release of the thigh cuff the internal and external diameters of the vein graft were measured at 30 s intervals for the first 2 min and again at 3, 4, 7 and 10 min. The laser-Doppler tracing confirmed the hyperaemic response in the skin capillaries. The patient then rested for at least 30 min before further investigations. The further investigations included monitoring the diameter of the vein graft after 400 μg GTN, given as sublingual spray, and assessment of measurement reproducibility.

Assessment of the effect of glyceryl trinitrate on vein graft stenoses

The details of the six vein graft stenoses which were assessed are given in Table 2. Following the detection of haemodynamically significant graft stenoses in the duplex surveillance programme, patients were admitted to hospital for further investigations and planned graft revision. Stenosis severity was assessed

from V2/V1, the ratio of the peak systolic velocity at the site of stenosis (V2) in comparison to the peak systolic velocity in the adjacent normal proximal vein graft (V1).[5] In six patients (Table 2), a GTN patch (10 mg, Nitro-Dur, Schering-Plough) was placed on the skin at the site overlying the graft stenosis. After 24 h the graft was reassessed by the same vascular technologist who had detected the stenosis the previous day (M.E).

Measurement errors

The reproducibility of arterial dimensions measured by ultrasonography has been investigated previously.[6] Similar methods were used to assess the repeatability[7] of vein graft diameters, with blinded observers measuring graft diameters on six patients on two separate occasions: for the external diameter of a vein graft in the adductor canal using a single observer the repeatability was 0.4 mm. Intraobserver variation in measurement of systolic velocity ratios (V2/V1) was investigated by repeated measurement on two vein graft stenoses. The position of the stenosis was identified by sharp changes in the colour-flow saturation. The peak systolic velocity at the site of maximal stenosis (V2) and at a normal proximal site (V1) were measured by the same observer on five occasions: for a mean velocity ratios of 2.5 and 5.8 the repeatability was 0.4 and 0.5, respectively.

Analysis of results

Results were compared using a Students *t*-test and are presented as mean $\pm$ S.D.

Results

Response of vein grafts to reactive hyperaemia

Parallel recordings were made of vein graft diameter and cutaneous capillary flow. These are shown for a single patient in Fig. 1. Peak vein graft external diameter was observed 120 s after release of the thigh cuff, whereas the increased flow in cutaneous capillaries was observed 30 s after release of the thigh cuff. This difference in the time of response was observed in all patients. The response of vein graft external diameter was very reproducible: in one patient, with the largest diameter vein graft, the hyperaemic test was repeated eight times and the mean results ($\pm$S.D.) are shown in Fig. 2. The resting and peak diameters for all patients are shown in Table 1: there was a significant increase to $112 \pm 1.9\%$ (mean $\pm$ S.E.M.) of the resting diameter 120 s after release of the occluding thigh cuff, $p = 0.026$, mean difference 0.81 mm. The diameter of the vein graft could be increased even further 5 min after sublingual GTN spray, when the external vein graft diameter had increased to $117 \pm 2.5\%$ (mean $\pm$ S.E.M.) of the resting diameter, $p = 0.007$, mean difference 1.16 mm (maximum diameters are given in Table 1). These results confirm that patent vein grafts can modulate their diameter in response to both flow and GTN.

Effect of GTN on flow through vein graft stenoses

The patients chosen for study had stenoses of varying severity, with V2/V1 ratios ranging from 2.5–6.3. Application of a GTN patch had no systematic effect on the peak systolic velocity proximal to a graft stenosis (Fig. 3a). Peak systolic velocity (V2) at the site of the vein graft stenosis fell in five of the six patients following GTN treatment, mean difference 0.8 ($p = 0.15$, Fig. 3b). Velocity ratio (V2/V1) was reduced in all six cases 24 h after application of a slow release GTN patch (Fig. 4). The mean velocity ratio before therapy was 4.6 ± 0.5 and fell to 3.2 ± 0.6 following GTN ($p = 0.04$, paired *t*-test). The reduction in velocity ratio was least for the two stenoses situated at distal sites in the vein grafts (patients 3 and 4, Fig. 4).

Discussion

The success of saphenous vein as an arterial bypass conduit may depend in part on the ability of the graft to simulate the responses of healthy arteries to variations in blood flow. In healthy arteries increased flow stimulates the endothelial synthesis of nitric oxide (endothelium-derived relaxing factor). Nitric oxide diffuses into the lumen and underlying smooth muscle, where it interacts with the active site of guanylate cyclase in platelets and vascular smooth muscle cells to limit platelet activation and effect smooth muscle cell relaxation, respectively.[8] Therefore dilatation is the normal response of arteries to increased flow. Similarly

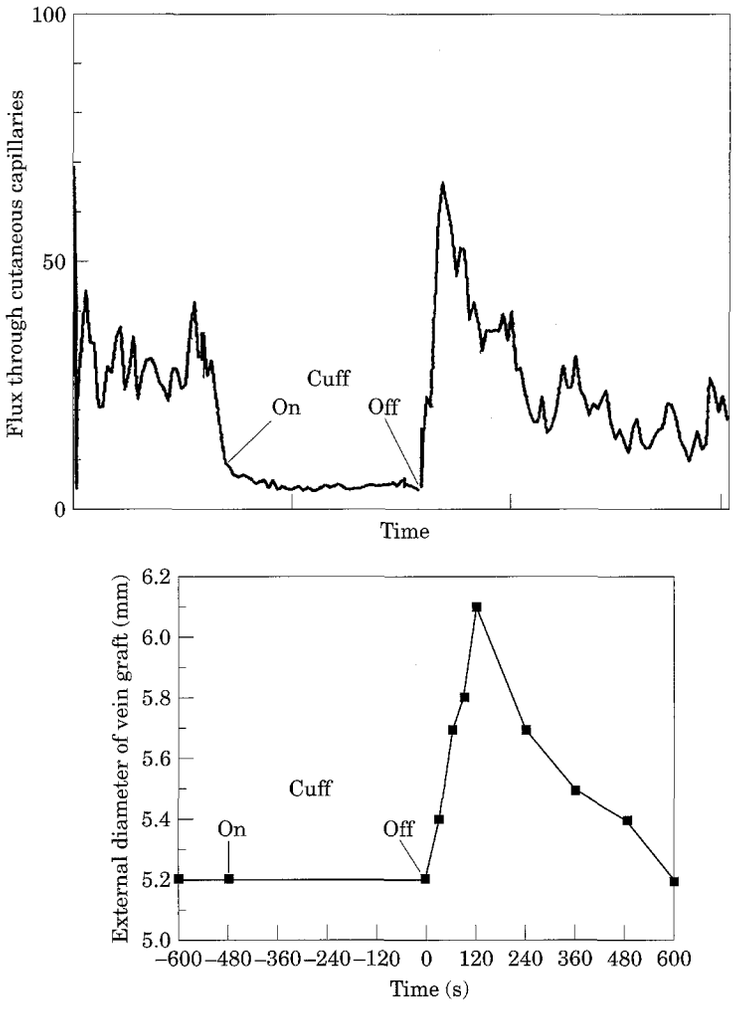

Fig. 1. Parallel responses of cutaneous capillaries and a vein graft to reactive hyperaemia. The upper panel shows the laser-Doppler recording of cellular flux in the cutaneous capillaries of the lateral calf, and the lower panel shows the change in external diameter of the vein graft in the adductor canal.

the nitrate family of drugs, of which GTN is a prototype, bypass the endothelium and act as nitric oxide donors which cause the relaxation of vascular smooth muscle. These responses have been explored elegantly *in vivo* by Deanfield and colleagues.[4] The superficial femoral artery increases to 109% of the resting diameter 90 s after the start of post-occlusive hyperaemia, and to 113% of the resting diameter after administration of sublingual GTN.[4] The production of endothelial nitric oxide is much lower in saphenous vein than in femoral artery, but the production of endothelial nitric oxide may be increased as one of the adaptations of saphenous vein to arterial flow.[9] Here we have shown that 6–9 months after implantation, patent saphenous vein grafts have developed responses to flow and GTN similar to those observed in healthy native arteries. Even diseased vein grafts, with haemodynamically significant stenoses, maintain some responsiveness to GTN.

The magnitude of both the endothelium-dependent (hyperaemic) and endothelium-independent (GTN) dilatation responses we observed in vein grafts were greater than those observed in femoral arteries. There are several reasons that might contribute to the magnitude of the observed responses in 6–9-month-old vein grafts. Firstly, these grafts are "new" to the arterial circulation; and both endothelium-dependent and endothelium-independent responses of femoral and brachial arteries are greater in children than in adults.[4] Secondly, we took considerable care to exclude smokers. Smoking is an important factor associated with endothelial dysfunction in both the femoral artery and saphenous vein and could dampen the response to reactive hyperaemia.[4,10] Thirdly, in vein grafts the maximum diameter was not reached until 2 min after release of thigh cuff occlusion or until 3–5 min after sublingual GTN, whereas previous studies have monitored femoral artery diameter 90 s after release of the occlusive thigh

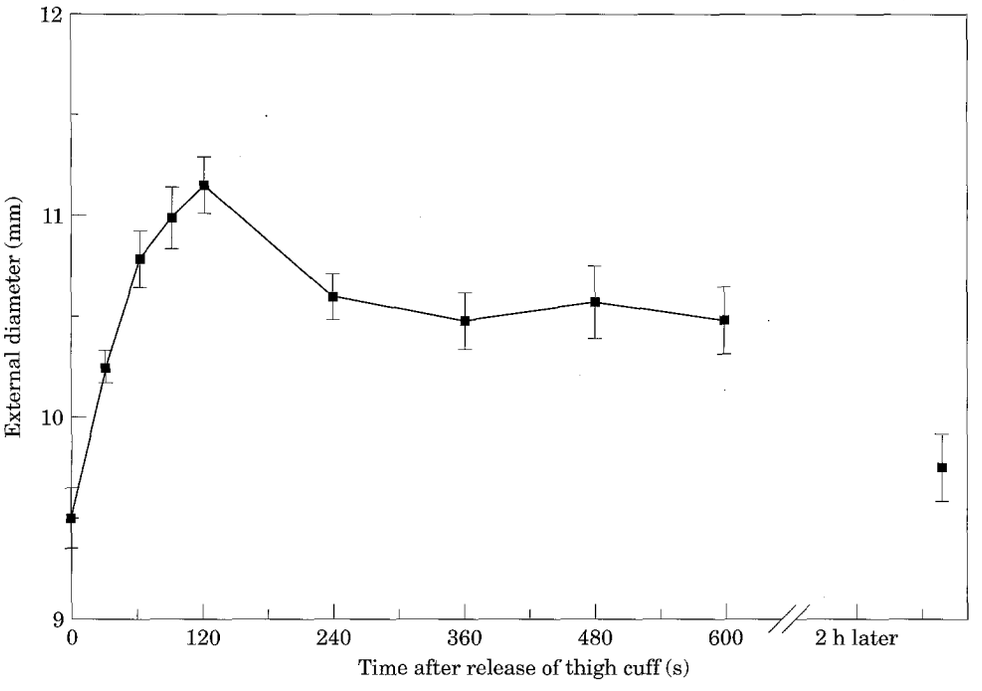

Fig. 2. Reproducibility of the dilatation of vein grafts in response to reactive hyperaemia. The hyperaemic response test was performed eight times by a single observer. Maximum dilatation was observed after 120 s. Results are shown as mean ± S.D.

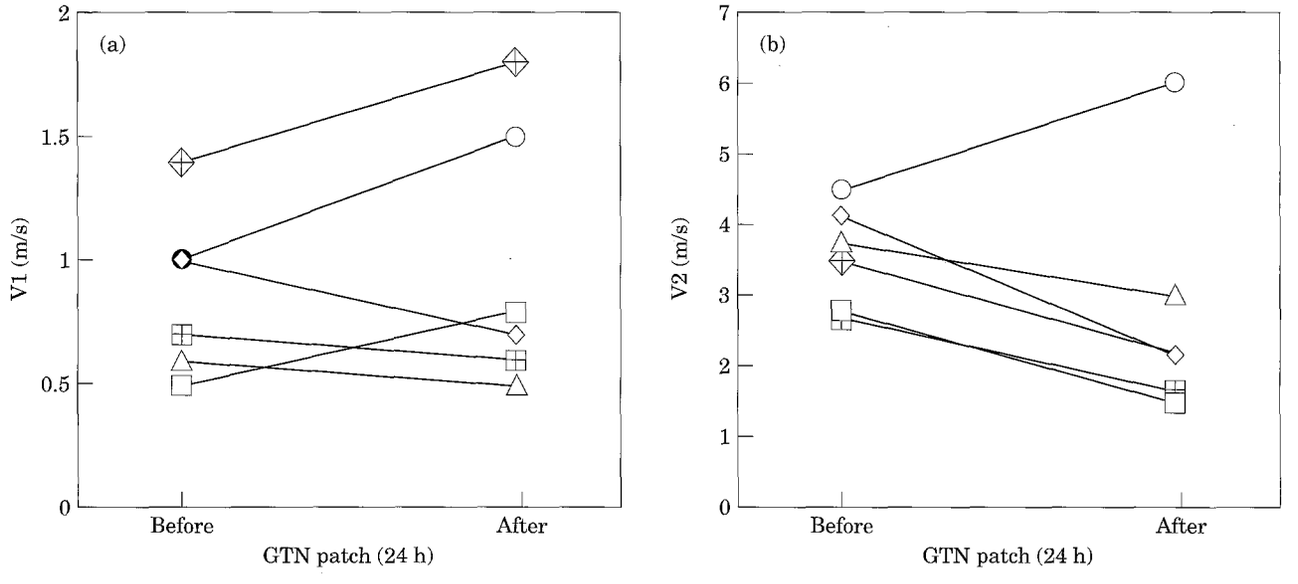

Fig. 3. Peak systolic velocities in the failing vein graft before and after the application of GTN patches. In (a) the peak systolic velocity proximal to the stenosis is shown, whereas in (b) the peak systolic velocity at the stenosis is shown.

cuff.[4] Interestingly, the peak hyperaemic response in cutaneous vessels was observed within 1 min of release of the occlusive thigh cuff (Fig. 1). Similar biological mechanisms are thought to control flow-stimulated vasodilatation in capillaries and arteries. The slower response of arteries and vein grafts might be attributed to their thicker walls, with intimal thickening providing an additional diffusion barrier for nitric oxide in vein grafts. The maximum increase in vein graft diameter, 17%, after GTN translates into a 37% increase in lumenal area.

Previous *in vitro* studies of excised vessels have indicated that there may be differences in the endothelium-dependent responses of aortocoronary and infrainguinal vein grafts.[2,3] Ku *et al.* showed that endothelium-dependent responses were present in patent aortocoronary vein grafts from patients undergoing

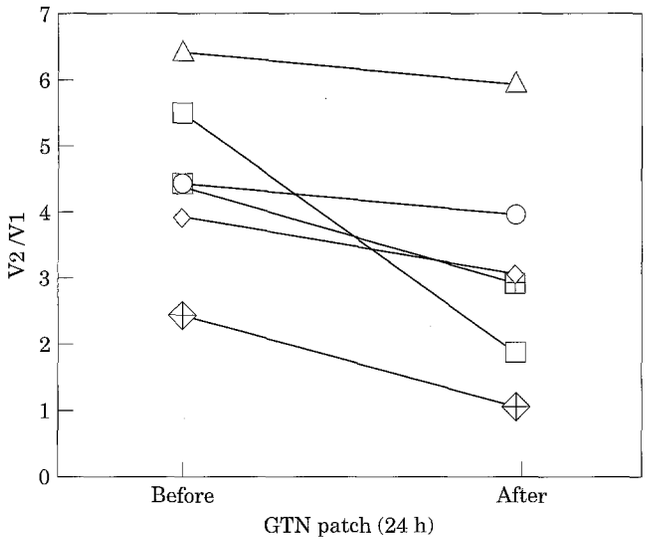

Fig. 4. The influence of GTN patches on the velocity gradient across vein graft stenoses. The GTN patches were applied for 24 h.

cardiac transplantation.[2] We have shown that endothelium-dependent responses are observed in infrainguinal vein grafts *in vivo*, whereas Park *et al.* failed to demonstrate these responses *in vitro* using segments of vein removed at revision of failing bypasses.[3] Many different factors cause endothelial dysfunction and some of these, particularly smoking, are the same factors associated with vein graft occlusion and failure.[10,11] This might provide some explanation for the contradictory results obtained, with the poor endothelium-dependent responses in failing infrainguinal vein grafts[3] being attributable to smoking, hypertension, hypercholesterolaemia and other factors associated with endothelial dysfunction. Such studies underscore the possible associations between endothelial function and vein graft patency.

Even if some of the endothelium-dependent responses of the failing vein graft are considerably impaired, the endothelium-independent responses, which include dilatation in response to GTN, may be better preserved. Certainly the local application of GTN appears to have been beneficial in our small series of patients with vein graft stenosis. Nitrates have both systemic and local effects: we chose the GTN patches for their slow release properties, providing a sustained dose with minimal side effects. These patches had a significant effect: in all six patients the velocity ratio across the graft stenosis was reduced after 24 h of a GTN patch. Interestingly, the improvement was most marked for the four proximal graft stenoses. There are at least two possible explanations for this observation. Firstly, these grafts were all *in situ* and this disparity of responses could be explained if the proximal saphenous vein were more sensitive to nitrate-mediated dilatation, although there is little evidence in Table 1 to support this. Secondly, in experimental studies intimal hyperplasia and vasomotor dysfunction increases towards the distal anastomosis of the vein graft.[12] A much larger number of patients would be required to confirm the different responsiveness to GTN of proximal and distal vein graft stenoses.

In conclusion, we have shown that patent infrainguinal vein grafts exhibit both endothelium-dependent and endothelium-independent vasodilator responses. The endothelium-independent responses are preserved, at least in part, in failing vein grafts. The preservation of this response can be exploited to provide temporary palliation, using GTN patches, of vein graft stenoses between their detection and definitive treatment.

Acknowledgements

We thank David Nott and Alun Davies for permission to study their patients. The research was supported by the Charing Cross Hospital Trustees and the AFG Research Foundation.

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Accepted 23 December 1996