Human saphenous vein grafts explanted from the arterial circulation demonstrate altered smooth-muscle and endothelial responses

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Purpose: Animal models have been used to assess the function of vascular smooth muscle and endothelium of veins grafted into arterial circulation. The primary model consists of grafting the external jugular vein into the carotid artery of the rabbit. These studies suggest a selective increase in the responsiveness of the grafted veins to serotonin. However, in both human cardiac and peripheral vascular operations, the saphenous, not the jugular, is the vein most frequently used. Thus the propriety of the rabbit model is unknown.

Methods: Human saphenous veins and vein grafts were obtained from patients undergoing leg vein bypass graft revisions ($n = 8$). The reversed vein grafts were placed into arterial circulation for periods ranging from 4 to 26 months before removal (mean 16 months). All vessels were immediately cut into rings and suspended in organ chambers for recording isometric contractions to norepinephrine and serotonin.

Results: The maximal contractions elicited by both norepinephrine and serotonin were reduced in human vein grafts in comparison to the results in human saphenous vein (maximal response to norepinephrine 1.42 ± 0.34 gm [vein graft] vs 4.59 ± 1.13 gm [saphenous vein], $p = 0.031$; maximal response to serotonin 2.68 ± 0.58 gm [vein graft] vs 4.72 ± 1.11 gm [saphenous vein], $p = 0.042$). Human vein grafts were less responsive to norepinephrine than was saphenous vein (negative log of concentration that caused 50% of the maximal response −5.91 ± 0.10 and −6.84 ± 0.22, respectively; $p < 0.009$). After precontraction with norepinephrine (to 30% of the maximal response), saphenous vein, but not vein grafts, demonstrated endothelium-dependent relaxation to acetylcholine (maximum relaxation 27.4% ± 6.8%; $p = 0.001$).

Conclusions: Human saphenous veins grafted into arterial circulation exhibit loss of endothelium-dependent relaxation to acetylcholine and diminished contractions to agonists (norepinephrine and serotonin). In contrast to rabbit data, serotonin elicits dose-dependent contractions in both human saphenous vein and human vein grafts. Since the vascular wall contractility varies widely across species, the relevance of rabbit vein graft data to human bypass grafts is uncertain. (J VASC SURG 1993;18:61-9.)

Nearly a half century after the introduction of the femoropopliteal bypass by Kunlin, autogenous saphenous vein remains the preferred conduit for infrainguinal revascularization. Both reversed vein bypass and in situ vein bypass techniques produce primary 5-year patency rates of 75% to 85%.[1,2] A disturbing number of vein grafts, however, continue to fail, leading to limb amputation in a number of patients. In recent years considerable research efforts on graft failure have focused on intimal hyperplasia and vascular contractility. Most of the research involving vein graft contractility and endothelium-dependent relaxation has been conducted in organ chamber experiments with vascular tissue from rabbits and dogs.[3-10] In the recent vascular surgery literature a model that uses New Zealand white rabbit external jugular vein anastomosed to common carotid artery in end-to-side fashion has been widely used for studying alterations in vessel wall physio-

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Table I. Patient data

Patient	*Age (yr)*	*Sex*	*Primary procedure*	*Duration of bypass (mo)*	*Location of HVG stenosis*	*Source of HVG (with respect to stenosis)*	*Source of vein for revision*	*DM*	*Smoking*	*HTN*
1	65	M	L femoropopliteal (RSV)	19	Proximal 1/3	Distal	Contralateral HSV	No	No	No
2	72	M	L femorotibial (RSV)	24	Proximal 1/3	Distal	Contralateral HSV	No	Yes	Yes
3	76	M	R femoropopliteal (RSV)	23	Proximal 1/3	Distal	Ipsilateral HSV	Yes	No	Yes
4	56	M	R popliteal-dorsalis pedis (RSV)	10	Proximal 1/3	Proximal	Ipsilateral HSV	Yes	No	No
5	65	M	R femoropopliteal (RSV)	4	Mid 1/3	Proximal	Contralateral HSV	No	Yes	Yes
6	61	F	L femoropopliteal (RSV)	26	Proximal 1/3	Distal	Arm vein	Yes	No	Yes
7	52	M	R femoropopliteal (RSV)	10	Distal 1/3	Proximal	Contralateral HSV	No	Yes	No
8	75	M	R femoropopliteal (RSV)	14	Proximal 1/3	Distal	Contralateral HSV	No	Yes	Yes

Mean age = 65 years; mean duration bypass grafts had been in place = 16 months.
DM, Diabetes Mellitus; *HTN,* hypertension; *L,* left; *R,* right; *RSV,* reversed saphenous vein.

logic function and vascular wall responsiveness to pharmacologic agents. With this model several authors have concluded that vein grafts in the arterial system lose endothelium-dependent relaxation to acetylcholine and develop de novo sensitivity to serotonin (5-HT).[3,4] In contrast, Ku et al.[11] removed human saphenous veins (HSV) grafted into coronary circulation from patients undergoing heart transplantation. In human saphenous vein grafts (HVG), acetylcholine, thrombin, histamine, and calcium ionophore produced endothelium-dependent relaxations. Although the differences between the rabbit data and human vein graft data were attributed to the different time periods that the veins were arterialized (4 weeks for rabbit vs 7 to 12 years for human), it appears equally likely that heterogeneity of endothelium-dependent responses between different species had a significant role.

To determine the physiologic basis of the contractility of saphenous veins grafted into the peripheral arterial circulation in humans, we have obtained saphenous veins and vein grafts from patients undergoing revision of patent but stenotic reversed femoropopliteal and femorotibial bypasses performed with reversed autogenous saphenous vein. The stenotic grafts were identified by a decrease in graft velocity detected by our routine postgrafting duplex surveillance program.[12] Organ chamber experiments were performed with the vascular ring segments obtained from these eight patients and the results are reported herein.

MATERIAL AND METHODS

Preparation of human vein grafts. Between August 1991 and January 1992, eight patients with vein graft stenosis were identified by duplex scanning graft surveillance and the suspected stenosis was confirmed in each patient by lower extremity arteriography. The initial lower extremity bypasses included six femoropopliteal bypasses, one femorotibial bypass, and one popliteal–to–dorsalis pedis bypass with reversed saphenous veins. All the anastomoses were constructed in end-to-side fashion with standard vascular techniques. These procedures were performed 4 months to 26 months before vein graft revision. The mean duration between grafting and revision was 16 months (Table I). The patients included seven men and one woman. The patients' ages ranged from 52 to 75 years with a mean age of 65 years. Five patients had a history of hypertension and three patients had diabetes. The location of the vein graft stenosis was in the proximal one third of the graft in six patients, middle one third in one, and distal one third in another. All vein graft revisions were performed with remaining segments of patent ipsilateral saphenous veins in two patients, contralateral saphenous veins in five patients, and an arm vein for interposition grafting in the remaining patient. The associated medical conditions and drug history of these patients were recorded.

With the approval of our institutional Human Research Committees, nonstenotic segments of vein graft adjacent to the stenotic area and undisturbed

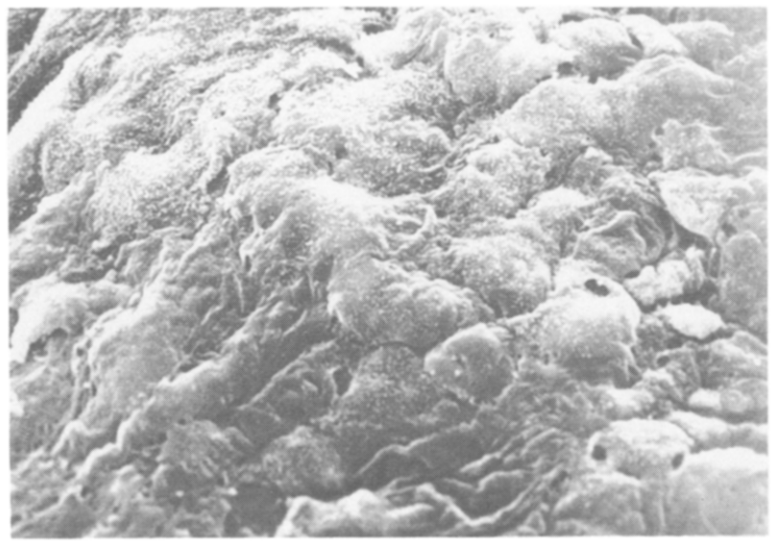

Fig. 1. Scanning electron micrograph of luminal surface of HVG. Note presence of normally appearing endothelial cells. (Original magnification ×1260.)

segments of saphenous vein were removed from each patient. The vein graft segments were taken from distal to the stenotic area in five patients and proximal to the stenosis in three. All the vessels were immediately placed in 4° C modified Krebs-Ringer bicarbonate solution of the following composition (millimolar concentrations): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; calcium ethylenediaminetetraacetic acid, 0.026; and glucose, 11.1 (control solution).

With the use of a dissecting microscope (Nikon 102, Tokyo, Japan; 10× magnification), the excised tissues were carefully cleaned of connective tissue and cut into segments 4 to 5 mm in length. There was no evidence of thrombosis in HVG or HSV. The endothelium was removed from one half of these excised segments with the use of dissecting forceps to gently rub the endothelium from the luminal surface of the rings. All the rings were then suspended in organ chambers filled with 25 ml control solution aerated with 95% O_2 and 5% CO_2 (pH 7.4, 37° C) and connected to force transducers (Grass FT-03; Grass Instruments, Quincy, Mass.). Changes in isometric force were recorded on a personal computer (PC's Limited 286[8], Austin, Texas) with interface hardware (Keithley Instruments, Cleveland, Ohio) and data acquisition software (CODAS; DATAQ Instruments, Inc., Akron, Ohio).[13]

At the beginning of each experiment, all transducers were calibrated. Each ring segment was stretched to its optimal tension (2.0 to 2.6 gm in HVG and 1.5 to 2.0 gm in HSV) for isometric contractions as determined by stepwise stretching and stimulation with 40 mmol/L KCl at each length. Mean basal tensions for HVG and HSV were 2.07 ± 0.09 gm and 2.09 ± 0.15 gm, respectively. After this procedure the rings were allowed to equilibrate for 30 minutes or more before the experiments were started.

Drugs. The following pharmacologic agents were used for the experiments: arterenol bitartrate (norepinephrine; Sigma Chemical Co., St. Louis, Mo.), 5-hydroxytryptamine (serotonin [5-HT]; Sigma), acetylcholine chloride (Sigma), cocaine hydrochloride (Sigma), hydrocortisone 21–sodium hemisuccinate (Sigma), and propranolol hydrochloride (Sigma). Concentrations of drugs are expressed as final concentrations in the organ chamber.

Experiments. At the beginning of each experiment all vascular rings were treated for 30 minutes with cocaine hydrochloride (5×10^{-6} mol/L), hydrocortisone 21–sodium hemisuccinate (3×10^{-5} mol/L), and propranolol hydrochloride (5×10^{-6} mol/L) to inhibit the neuronal and extraneuronal uptake of monoamines and β-adrenoreceptors, respectively. Maximal responsiveness to norepinephrine (10^{-4} mol/L) was measured under these conditions. Subsequent contractions with other agonists were expressed as percent of the maximal response to that particular agonist ($agonist_{max}$).

Concentration-response curves for norepinephrine and 5-HT were constructed in a cumulative manner by increasing the concentration of agonists in half-log increments from 10^{-9} to 10^{-4} mol/L.[14]

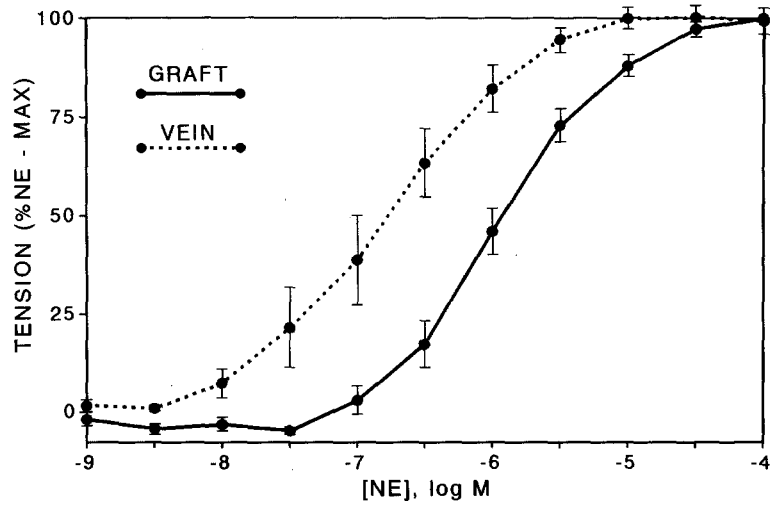

Fig. 2. Plot of norepinephrine *(NE)* concentration-response curves in HVG and HSV.

Between agents the organ chambers were rinsed with control solution until the vessels returned to baseline tension ($\geq 4\times$). Next, with 3×10^{-7} to 10^{-6} mol/L norepinephrine, 30% maximal contraction was achieved (NE EC_{30}). After stabilization at NE EC_{30}, acetylcholine was administered to the organ chambers in full-log increments from 10^{-9} to 10^{-4} mol/L. At the end of the experiments, vascular ring segments were placed in glutaraldehyde solution for electron microscopic examination to confirm the presence of endothelium in these segments.

Histologic study. Vein graft rings in glutaraldehyde solution were fixed in Ito-Karnovsky solution (mixture of glutaraldehyde and paraformaldehyde) for 1 hour. Next, vein grafts were washed in buffer solution for 20 minutes before they were fixed in 1% osmium tetroxide. Ethyl alcohol was used to dehydrate these vessels in graded series. After these segments were placed in 100% ethanol and freon 113, CO_2 was used to critically dry the specimens. A thin layer of gold (30 Å) was placed on the surface of the specimens before electron microscopic examination. Scanning electron microscopy was performed with AMR Model 1000 scanning electron microscope (Amray Inc., Bedford, Mass.). The photomicrographs demonstrated the presence of endothelium in the HVG (Fig. 1).

Statistics. Results of the experiments are expressed as means ± SEM. In all the experiments, n equals the number of individuals from whom vessels were taken. Concentration-response curves were compared by either the negative log of the concentration that caused 50% of maximal response (ED_{50}) or the maximal force of contraction for the particular agonist. Statistical evaluations of the HVG and HSV data were performed by use of Student's t test for paired observations. One patient did not have saphenous vein available for comparison, and the vein graft taken from this patient was not included in the statistical calculations ($n = 7$). Comparison of vein graft data between hypertensive and normotensive patients was performed with Student's t test for unpaired observations. Means were considered significantly different when $p < 0.05$.

RESULTS

Norepinephrine. Norepinephrine produced dose-dependent contractions in both HSV and HVG ($n = 7$, Fig. 2). The maximal contraction elicited by norepinephrine was significantly reduced in HVG as compared with that in HSV (NE_{max} = 1.42 ± 0.34 gm [HVG] vs 4.59 ± 1.13 gm [HSV], $p = 0.031$; Fig. 3). Additionally, HVG was less responsive to norepinephrine than HSV, with $EC_{50} = -5.91 \pm 0.10$ (HVG) and -6.84 ± 0.22 (HSV), respectively ($p = 0.009$, Fig. 2).

5-HT. 5-HT also produced dose-dependent contraction in both HSV and HVG ($n = 7$, Fig. 4). The maximal tension produced by HVG was significantly reduced as compared with that in HSV (5-HT_{max} = 2.68 ± 0.58 gm [HVG] vs 4.72 ± 1.11 gm [HSV], $p = 0.042$; Fig. 3). There was no difference in the sensitivity of this agonist between HSV and HVG.

Acetylcholine. After precontraction to NE EC_{30}, acetylcholine caused endothelium-dependent relaxation in HSV. This response was dose-dependent, and acetylcholine at 10^{-4} mol/L produced

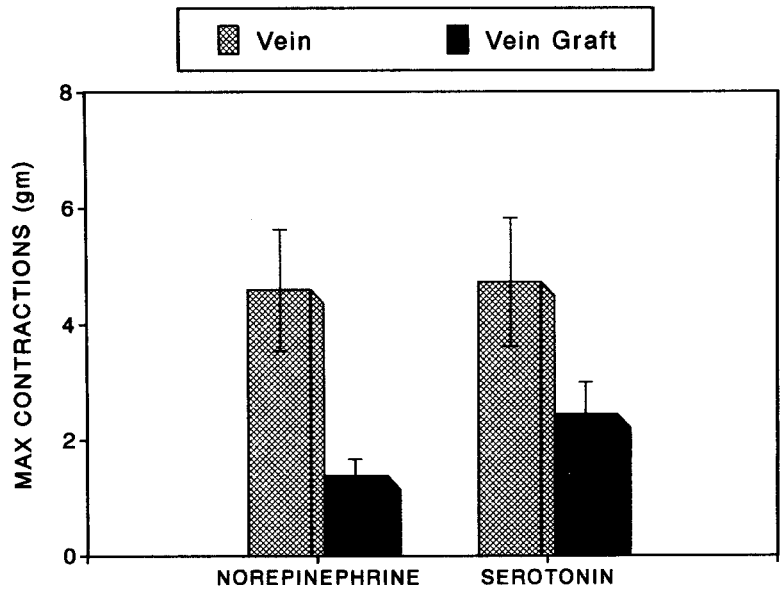

Fig. 3. Graph of maximal amount of tension elicited with norepinephrine and 5-HT in HVG and HSV.

27.4% ± 6.8% relaxation in HSV (n = 7, Fig. 5). In contrast, HVG did not exhibit relaxation to acetylcholine.

Without endothelium. As outlined in Table II, both HSV and HVG segments denuded of endothelium behaved in a similar manner to that of vessel segments with intact endothelium. There was no difference in maximal amount of tension elicited or EC_{50} values to either agonist (norepinephrine and 5-HT).

DISCUSSION

The results of our organ chamber experiments confirm previously published results demonstrating that HSV contracts in response to 5-HT in a dose-dependent manner.[13,15] Although vein grafts maintain their sensitivity to 5-HT, the maximal amount of tension produced by 5-HT is significantly reduced in HVG as compared with that in HSV. Similarly, the HVG have diminished maximal contraction to norepinephrine. HVG placed in the arterial circulation for a mean period of 16 months becomes less sensitive to norepinephrine as indicated by a significant shift of the concentration-response curve to the right. These changes are not endothelium-dependent and appear to reflect alterations in smooth-muscle responsiveness to norepinephrine in the vein grafts.

Despite the presence of structurally intact endothelium, the HVG demonstrate altered endothelial function. HVG appear to lose the ability to relax in response to acetylcholine, an action mediated by the endothelial release of endothelial-derived relaxing factor. This loss of endothelium-dependent function has been previously demonstrated in the rabbit external jugular veins, but not in HVG.[3,6]

Our human vein graft data differ from the rabbit data in several ways. For example, the rabbit data suggest that the development of serotonergic receptors may predispose vein grafts to vasoconstriction. In contrast, the present study indicates that 5-HT produces consistent dose-dependent contractions in both HSV and HVG. Although the finding of 5-HT–induced contractions of HSV has been published previously, the diminished maximal contractions to 5-HT in HVG as compared with those in HSV has not been reported previously. In humans the diminished responsiveness to 5-HT makes HVG less susceptible, not more susceptible, to vasoconstriction than HSV. Thus it is quite unlikely that 5-HT has a significant role in vein graft vasoconstriction. This marked difference in responsiveness to 5-HT between the HSV and the rabbit external jugular veins is certainly one of the reasons the rabbit external jugular vein is not the optimal vessel for studying human vein graft physiology.

In regard to another agonist, norepinephrine, our human data support the previously published rabbit data that vein grafts are less responsive to norepinephrine. However, in rabbits, the vein grafts re-

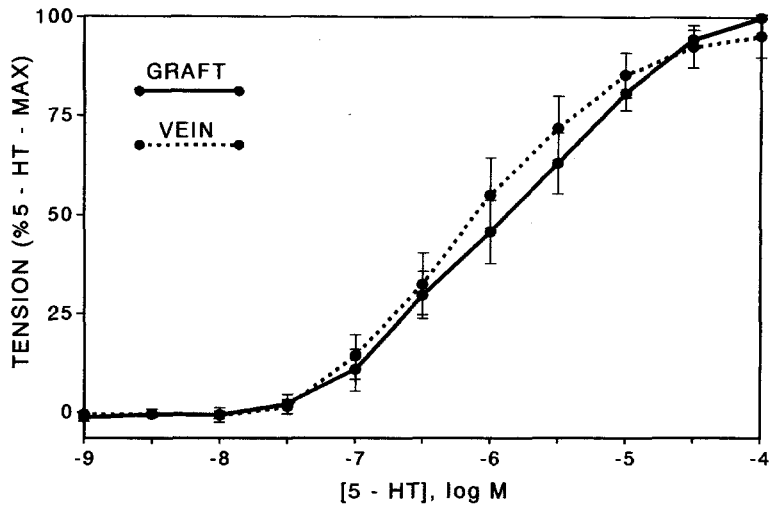

Fig. 4. Plot of 5-HT concentration-response curves in HVG and HSV.

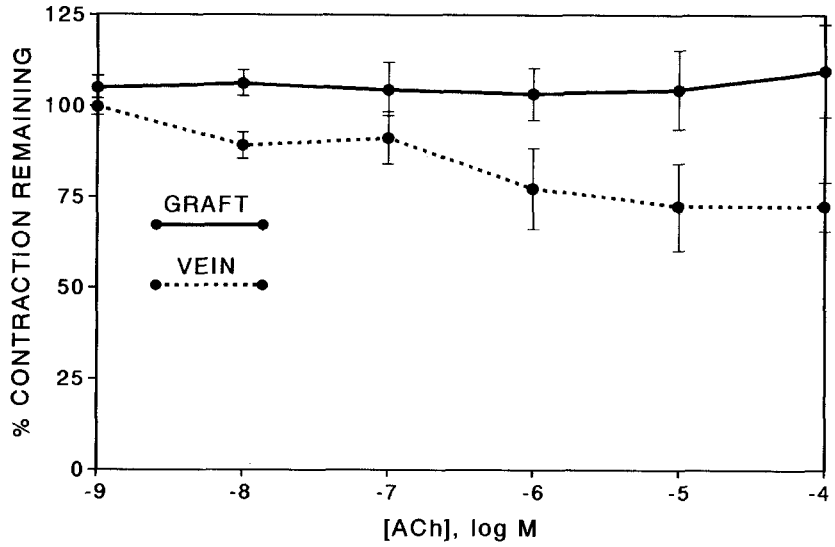

Fig. 5. Plot of concentration-response curves to acetylcholine *(ACh)* in HSV and HVG after precontraction to NE EC_{30}.

moved from hypertensive animals were more sensitive to agonists (norepinephrine, histamine, angiotensin, and 5-HT) than were those taken from normotensive animals. Interestingly, when HVG data are divided between hypertensive (n = 5) and normotensive (n = 3) patients, there are no significant differences between these two groups in ED_{50} values or maximal contractions to either norepinephrine or 5-HT. Although our data contradict the rabbit data, the number of vein grafts in our subgroups is not large enough for us to exclude type II error.

The endothelium-dependent function of vein graft is another area that has been extensively studied. Animal experiments with both rabbits and dogs have established that vein grafts lose the endothelium-dependent relaxations produced by acetylcholine. Scanning electron microscopy has confirmed the structural presence of endothelium in the animal grafts, as well as in our human grafts. Therefore loss of acetylcholine-induced relaxation in vein graft is due to a functional change in the endothelium and not to the loss of endothelium itself. Because acetylcholine induces production of endothelial-derived relaxing factor (nitric oxide), which promotes smooth-muscle cell relaxation by increasing

Table II. HVG and HSV (endothelium versus no endothelium)

	Vein grafts			*Saphenous veins*		
	Endothelium	*No endothelium*	*p Value*	*Endothelium*	*No endothelium*	*p Value*
Norepinephrine						
NE_{max} (gm)	1.42 ± 0.34	1.38 ± 0.34	0.94	4.59 ± 1.13	4.96 ± 1.68	0.86
EC_{50}	−5.91 ± 0.10	−5.96 ± 0.15	0.79	−6.84 ± 0.22	−6.74 ± 0.34	0.81
5-HT						
5-HT_{max} (gm)	2.68 ± 0.58	2.21 ± 0.55	0.57	4.72 ± 1.11	5.09 ± 1.69	0.86
EC_{50}	−5.85 ± 0.20	−5.81 ± 0.18	0.88	−6.01 ± 0.26	−6.25 ± 0.21	0.49

cyclic guanosine monophosphate, Komori et al.[10] measured levels of cyclic guanosine monophosphate in both rabbit vein grafts and veins to further define the mechanisms responsible for the impaired response to acetylcholine observed in vein grafts. They noted diminished levels of cyclic guanosine monophosphate production in rabbit vein grafts in comparison to the levels in control veins after stimulation with acetylcholine. Additionally, nitric oxide and nitric oxide donor were able to directly evoke relaxation in vein grafts denuded of endothelium. Thus these data suggest that production of endothelium-derived nitric oxide is impaired in vein grafts and primarily responsible for diminished response to acetylcholine seen in rabbit vein grafts.

Contrary to animal data, Ku et al.[11] reported that HVG placed in the coronary circulation maintain their responsiveness to acetylcholine. Unlike the previous animal experiments, these HSV were placed into the arterial circulation for a mean duration of 4.4 years. It was hypothesized that the short period of time involved in the animal vein graft studies (4 to 6 weeks) did not allow for the return of endothelial function seen in HVG. Interestingly, the results of our HVG study directly contradict the data of Ku et al.[11] Although our saphenous vein grafts had been placed into arterial circulation for a mean of 16 months, vein grafts nonetheless demonstrated loss of endothelium-dependent relaxation to acetylcholine. Perhaps HVG placed in coronary circulation behave differently from vein grafts placed in peripheral circulation. At present, the cause of this discrepancy is unclear.

In summary, despite the significant insights we have gained regarding physiologic alteration of vein grafts placed in the arterial circulation in animal models, there is much conflicting data regarding smooth muscle and endothelial cell function in vein grafts. This difference is accentuated not only by the type of vessels studied but also clearly by the animal model chosen for experimentation. Thus future research efforts on vein graft physiology should be directed toward HSV.

We appreciate the technical assistance from Mike Webb at the Oregon Primate Research Center in performing the scanning electron microscopy for our experiments.

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DISCUSSION

Dr. Richard L. McCann (Durham, N.C.). Dr. Park and his colleagues have undertaken a scientific study of the vasoreactivity of eight HVG left in the peripheral circulation for periods ranging from 10 to 26 months and compared the results to the reactivity of seven or eight freshly explanted HSV segments.

The basic findings of this study were that maximum in vitro–generated active tension in vein grafts in response to norepinephrine and 5-HT was reduced compared with that in fresh veins, that vein grafts were less sensitive to norepinephrine than fresh veins, that vein grafts were equally as sensitive to 5-HT as were fresh veins, and that acetylcholine-induced relaxation is reduced from 25% in fresh veins to nil in vein grafts. The data regarding fresh saphenous veins agree well with previously reported results from our own and other laboratories.

The authors suggest that in light of their findings the relevance of the rabbit jugular vein graft model should be questioned, inasmuch as their responses differ significantly from the current series. Careful examination of the available rabbit data, however, shows that the results agree much more than they disagree. In response to grafting, rabbit jugular veins exhibit profoundly decreased maximal tension in response to several agonists including norepinephrine, decreased sensitivity to norepinephrine, and complete unresponsiveness to the relaxant properties of acetylcholine. The only apparent difference between rabbit and HVG is the curious finding of increased maximal tension in sensitivity to 5-HT in rabbit vein grafts compared with that in control external jugular veins, the significance of which has not been determined.

There are a number of questions that I would ask the authors to address.

The specimens were removed from grafts that had critical stenoses. In what way would vasoreactivity of a stenotic vein graft be expected to differ from that of the more normally functioning grafts?

No data on histopathologic examination are presented. Is there any correlation between vasoreactivity and the histologic appearance of the vein graft segments that were studied?

No data are presented on the deendothelialized rings. What was the purpose of this and what was learned from studying these segments?

What is the proposed mechanism for the decreased maximal tension and decreased sensitivity to norepinephrine?

There are data to suggest that increased wall tension in grafted segments is responsible for attenuation in contraction as smooth muscle cells are acutely or chronically stretched. Would the authors agree with this hypothesis?

The authors' abstract previously presented at the Federation of American Societies for Experimental Biology (FASEB) meeting included eight of each of the control veins and vein grafts and yet the present report includes only seven control veins. Despite this difference, the data presented are exactly the same. Could the authors account for this apparent discrepancy?

Finally, it seems a little sweeping to me to dismiss all data obtained from experimental models on the basis of seven or eight observations of possibly diseased vessels.

Dr. Thomas C. Park. At our institution we routinely perform vein graft revision by replacing the stenotic segment with a new vein interposition graft. With this method we were able to remove segments of normal-appearing vein graft adjacent to the stenotic areas, and these were used for our experiments. During the experiment these vein graft segments were inspected with a dissecting microscope to make sure that there was no evidence of thrombosis or stenosis.

When the deendothelialized segments were compared with the endothelium-intact vein grafts, there were no differences between these two groups' response to 5-HT or norepinephrine.

I think the role vein graft reactivity has in vein graft failures, if there is one at all, is unknown. However, the group from Duke has reported in vascular surgery literature, solely on the basis of rabbit data that development of 5-HT receptors in the veins grafted into the arterial circulation may lead to vasoconstriction, ultimately leading to graft failure. Unfortunately, the role of vasospasm or vasoconstriction has never been clearly established to be significant in vein graft failures. In addition, our human data contradict the rabbit data. HVG have diminished

response to 5-HT as compared with that in the veins. Therefore this difference in response to 5-HT between HSV and rabbit external jugular vein is a clear example of why these two models are not parallel.

Dr. Christopher K. Zarins (Chicago, Ill.). Would you care to comment on the FASEB abstract and the numbers in that?

Dr. Park. At the FASEB meeting we presented our preliminary data in a poster session. When we initially presented our data, we used $n = 8$ because we had eight vein grafts. However, only seven patients had saphenous veins corresponding to the vein grafts. As these data were paired, we dropped the unpaired vein graft in our data analysis as outlined in the statistical section of this manuscript. Unfortunately, this error was not detected in the earlier FASEB presentation.

Dr. Zarins. But were the numbers the same?

Dr. Park. The data in the presentation and the manuscript reflect the calculations performed with the paired t test. Although the p values were recalculated, the values were still significant.

Dr. Daniel B. Walsh (Lebanon, N.H.). I gather that the saphenous vein that you used as control was probably part of some saphenous vein that had been used as a bypass graft previously and thus it was an end of a saphenous vein. Were these patent? I think this is important because these veins may have had a higher or lower thromboxane production. And I am not sure the endothelium in a transected saphenous vein is the same as in saphenous vein in situ in a normal leg.

Dr. Park. In our vein graft revisions, we have often used contralateral saphenous veins. In patients who have had incomplete removal of saphenous vein from the ipsilateral leg, we have only used a patent segment or a patent branch of the main saphenous vein for our experiments. As these veins in our experiments were patent, I would assume that the endothelial function and thromboxane production should be same as in a normal vein.

Dr. Ronald L. Dalman (Stanford, Calif.). Did any of these patients receive nitrous oxide as part of the anesthetic technique? And if so, how would that affect determining ACh-dependent relaxations?

Dr. Park. We did not collect the data on the types of anesthetic used in these patients. Because our human vein grafts were compared with the saphenous veins taken from the same patient, the type of anesthetic used did not account for the differences noted between veins and vein grafts in our data.

Dr. Zarins. Dr. McCann asked you about the histologic features of these veins. These were failing vein grafts. Were they thickened and sclerotic? Do you have any data on that?

Dr. Park. In regards to the histologic data, we have only examined the presence of endothelium with scanning electron microscopy. The emphasis of the manuscript was on the functional aspects of human vein grafts. The histologic alterations in vein grafts have been clearly correlated to vasoreactivity in animal models, and these data have been already reported by other authors. Therefore we did not address the histologic aspects of the vein grafts in our study.