

# Functional and Morphologic Assessment of Saphenous Veins Harvested with Minimally Invasive Techniques Using a Modified Laryngoscope

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## ABSTRACT

**Background:** Minimally invasive saphenous vein harvesting techniques have been shown to reduce postoperative morbidity. Commercially available and often disposable instruments add significant costs to the operation. To lower expenses and to reduce postoperative morbidity, we used an ordinary laryngoscope fitted with a modified #3 Heine™ blade for harvesting the greater saphenous vein for coronary artery bypass surgery.

**Objective:** To assess the integrity and function of the autologous, undistended, long saphenous vein harvested by a modified laryngoscope.

**Methods:** Morphology was examined by light and scanning electron microscopy. Endothelial function was assessed by vascular reactivity in an isolated organ bath. Veins, randomly taken and prepared traditionally, served as a control group. Contractile function was measured in response to potassium chloride. Endothelium-dependent relaxation was assessed by use of acetylcholine and calculated as percentage relaxation.

**Results:** There were no significant differences, in response to the constricting or dilating agent, in vein rings taken with the modified laryngoscope compared with the traditional "open" technique ( $n = 10$ ,  $p > 0.05$  by ANOVA). Histologic examination by light and scanning electron microscopy showed no significant damage to the endothelial layer.

**Conclusions:** Minimally invasive saphenous vein harvesting, using a modified laryngoscope yields morphologically and biologically intact veins.

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## INTRODUCTION

Conventional harvesting of the greater saphenous vein results in a long incision ranging as far as from the medial ankle up to the sapheno-femoral junction. Complications associated with this procedure include hematoma, seroma, edema, infection, and skin necrosis, with an incidence of up to 44% [DeLaria 1981, Wipke-Tevis 1996]. A number of minimally invasive techniques using commercially available instruments such as a Richardson retractor [Slaughter 1998] or a laryngoscope [Stavridis 1998] have been used to reduce postoperative morbidity and scarring, as well as to lower costs by reducing both length of hospital stay and medication. We assessed biologic and morphologic properties of veins taken by a previously described, inexpensive, minimally invasive technique [Stavridis 1998], but used a conventional laryngoscope fitted with a modified blade.

## MATERIALS AND METHODS

### Patients

Forty-three patients, scheduled for coronary artery bypass grafting, had their greater saphenous vein harvested using a laryngoscope fitted with a modified blade (Figure 1, ). Patients suffering from peripheral vascular disease or extensive varicosities were excluded.

### Technique

A 2.5-cm long incision was made 15 cm above the medial ankle and a short segment of saphenous vein was prepared under direct vision. We then used a #3 laryngoscope (Heine™, Herrsching, Germany) with a modified blade. The tip of the blade was narrowed to facilitate insertion and enhance blunt separation of the perivascular tissue compared with the originally shaped blade. The blade was gently inserted into the anterior perivascular tissue. The vein was then prepared under direct vision using con-



Figure 1. Modified blade of laryngoscope.



Figure 2. Incisions shown after saphenous vein harvesting.

ventional preparation scissors within the illuminated tunnel created by retracting the blade. Major side branches were easily identified, clipped, and cut using a clipapplier (Surgiclip; Autosuture™, Tönisvorst, Germany). We intentionally did not use any cautery so that clinical postoperative outcome was not influenced. Depending on the calf's or thigh's length, 2 to 3 incisions were made (Figure 2, ⊙) by avoiding the popliteal fossa, and the vein was prepared bidirectionally through the tunnel that was created. Dissecting the vein in the popliteal fossa always proved to be very challenging due to the numerous branching tributaries. Avoiding an incision at this anatomical landmark improves postoperative pain. The leg was abducted when dissecting the vein in the calf towards the sapheno-femoral junction. One surgeon performed the entire saphenous vein harvesting procedure. Having prepared the required length of vein, it was clipped at its proximal and distal ends and was carefully taken out through the "tunnel". Any side branches previously missed were ligated *ex vivo*. Skin incisions were closed with 4-0 prolene using no drainage system. The leg was then wrapped with an elastic bandage for 24 hours.

#### Organ bath

During and after harvesting, no infusion for rinsing or distending was used. A 2-cm non-distended vein segment was taken and immediately stored in aerated Krebs-Henseleit solution at 4°C and then taken to the lab within 5 minutes. The composition of Krebs-Henseleit solution was as follows (mmol/L): Na<sup>+</sup>, 144; K<sup>+</sup>, 5.9; Ca<sup>2+</sup>, 2.5; Mg<sup>2+</sup>, 1.2; Cl<sup>-</sup>, 128.7; HC0<sub>3</sub><sup>-</sup>, 25; SO<sub>4</sub><sup>2-</sup>, 1.2; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 1.2; and glucose, 11.

Having carefully prepared the vein out of its surrounding tissue, the segment was cut into 2 equal rings of an approximate 3-mm width. The vein rings were mounted between two hooks immersed in a 5-ml organ bath containing Krebs-Henseleit solution, as previously described [He 1988]. The solution was maintained at a temperature of 37°C and gassed with 95% oxygen and 5% carbon dioxide. The upper hook was controlled by a micrometer connected to a strain gauge linked to a recorder and computer (Technical and Scientific Equipment, Inc., Bad Homburg,

Germany), thereby allowing the measurement of wall force and length (internal circumference) of the venous ring. The venous rings were exposed to a defined resting tension and thereafter stretched in steps to a standard point on the vein's individual length-passive wall tension curve. The internal radius (R) of each venous ring could be determined by iterative fitting of pressure-dependent isobars intersecting the exponential length-tension curve by a computer software program (Technical and Scientific Equipment, Inc., Bad Homburg, Germany). Depending on each vein's individual length-tension curve, the stretching force was stopped when a transmural pressure of 60 mm Hg was reached, thereby defining the internal radius (R100).

Transmural pressure was set at 60 mm Hg instead of 20 or 50 mm Hg because pilot studies showed that reactivity was clearer at this setting. The rings were then released to 90% of their internal circumference. The isometric force at this setting has been termed the "passive" or "resting" force in the absence of any constrictor tone. After equilibrating the rings for 1 hour, 2 x 10<sup>-4</sup> M potassium chloride (KCl) was added to each organ bath and contracting wall force was read by the recorder. When a steady state was reached, 4 x 10<sup>-6</sup> M acetylcholine was added to induce relaxation. Normal saline solution was used as a reference in an additional bath. Relaxation was expressed as percentage relaxation of the post-contraction wall force induced by KCl and acetylcholine that were used were tested in pilot experiments and were in accordance with the literature.

#### Histology

Ten randomly taken samples of 1-cm width were stored in 10% buffered formaldehyde for light microscopy and 4% buffered glutaraldehyde for scanning electron microscopy. A pathologist, blinded to the procedure, studied the specimens on the basis of morphologic and descriptive criteria as previously described [Gundry 1980], such as endothelial cell separation, cell loss, exposed basement membrane, and medial edema, in addition to intimal fractures. For comparison, 10 vein rings of 1-cm width from traditionally harvest-

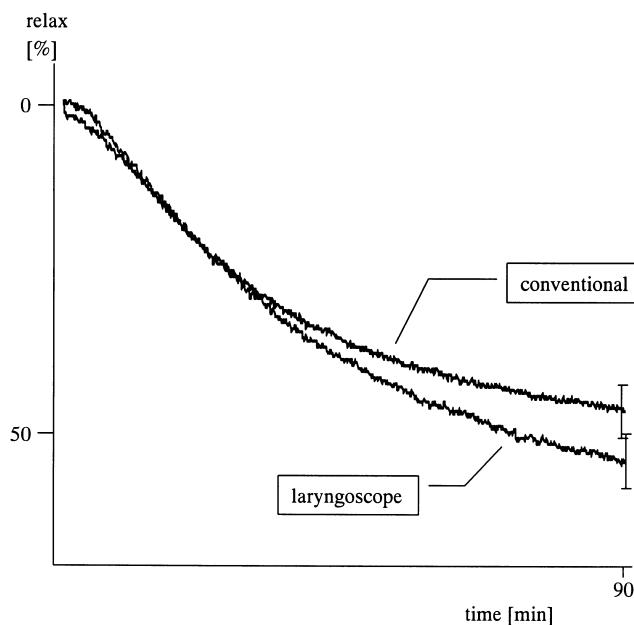


Figure 3. Relaxation after exposure to  $4 \times 10^{-4}$  acetylcholine of vein rings harvested conventionally ( $n = 10$ ) or using a modified laryngoscope ( $n = 10$ ).

ed veins were taken and their morphologic and functional properties assessed, as mentioned above.

#### Data analysis

Cumulative dose-response curves were used because we wished to only evaluate simple reactivity. "Reactivity" throughout the text describes a response in terms of the maximum relaxation (or contraction) to the pharmacological vasodilator (or vasoconstrictor), expressed as a percentage of the precontracted (or predilated) force, regardless of location on the response curve. All statistical data presented in the Figures and Tables are means  $\pm$  SEM. The statistical significance of possible differences in quantifiable variables (contraction and relaxation in millinewtons [mN]) was derived by analysis of variance (ANOVA). Statistical significance was defined as  $p$  values  $< 0.05$ .

## RESULTS

#### Organ bath

The traditionally harvested veins showed a radius of  $920 \pm 18 \mu\text{m}$ . All samples tested ( $n = 10$ ) showed reactivity to  $2 \times 10^{-4}$  M KCl with a resulting contraction force of  $16.4 \pm 0.4$  mN. Application of  $4 \times 10^{-6}$  M acetylcholine resulted in an average relaxation of  $8.1 \pm 0.8$  mN, thereby relaxing the rings by an average of  $49.2 \pm 4.9\%$ . Exposure to the saline solution resulted in a relaxation of  $0.9 \pm 0.1$  mN ( $5.4 \pm 0.6\%$ ).

The veins harvested via minimally invasive techniques showed a radius of  $914 \pm 16 \mu\text{m}$ . Reactivity to  $2 \times 10^{-4}$  M KCl was observed in all samples with a contraction force of  $16.1 \pm 0.3$  mN. Application of acetylcholine ( $4 \times 10^{-6}$  M)

Table 1. Responder rate (RR), force of contraction induced by KCl, and relaxation induced by acetylcholine: All showed no significant differences between modified laryngoscope group (L) and control group (C)

Group (n = 10)	RR, %	Force of Contraction (mN)	Relaxation (mN)	%
L	100	$16.4 \pm 0.4$	$8.1 \pm 0.8$	$49.2 \pm 4.9$
C	100	$16.1 \pm 0.3$	$9.3 \pm 0.8$	$57.6 \pm 5.0$
Statistic		NS	NS	NS

relaxed the vein rings by an average of  $9.3 \pm 0.8$  mN ( $57.6 \pm 5.0\%$ ) and exposure of the segments to saline solution resulted in a relaxation of  $1.2 \pm 0.2$  mN ( $7.4 \pm 11.2\%$ ). Resulting graphs, reflecting the application of acetylcholine, are shown in Figure 3 (◎) and values are shown in Table 1 (◎).

#### Histology

In all samples of both groups the overlying endothelial layer appeared well preserved and was not fissured. The underlying basement membrane was not exposed and there were no adherent red blood cells or platelets to suggest denudation or thrombogenic surfaces. Minor lineation of less than 10 cells was encountered in 4 out of 10 samples and no major lineation of more than 10 endothelial cells was seen in the minimally invasive group. In the conventional group, minor lineation of fewer than 10 cells was encountered in 5 of 10 samples and no major lineation of more than 10 endothelial cells was seen. Discrete endothelial edema was detected in almost all of the samples of both groups suggesting a phase of hypoxia.

## DISCUSSION

Mayo was first to describe a semi-closed technique for the harvesting of the saphenous vein in 1906 [Mayo 1906]. Since then, various new minimally invasive techniques have been described using commercially available instruments. However, employing new techniques for vein harvest may be harmful to the conduit. Preserved structural and functional integrity of the conduits used for bypass is essential for long-term graft performance [He 1989, Dhein 1991]. Factors which impair the endothelial layer occur during [Gundry 1980, Angelini 1989] or after surgical preparation when the graft is distended [Bonchek 1980, Hasse 1981] or stretched [Bush 1986].

We have demonstrated that harvesting the vein with the modified laryngoscope compromises neither the morphologic integrity nor vascular reactivity. The clinical applicability of this procedure has been demonstrated before [Stavridis 1998] and has been confirmed by our experiences. However there is, as yet, no experience with long-term patency compared with the traditional "open" technique".

In conclusion, this inexpensive procedure using a laryngoscope with a modified blade has proven to be easily applied (after a learning curve of 5 to 10 veins). The harvested veins were shown to be intact both morphologically and functionally, thus demonstrating the safety of the procedure. We believe this technique is a suitable and inexpensive alternative to other minimally invasive techniques for saphenous vein harvesting.

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