

# The effect of distension pressure on endothelial injury and vasodilatation response in saphenous vein grafts: conversion of a bypass graft to a dead pipe

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## Abstract

**Introduction:** Endothelial damage caused by high pressure applied for spasm relaxation during graft preparation is one of the most plausible theories explaining early graft failure.

**Aim of the study:** We aimed to demonstrate the extent of endothelial damage in saphenous vein grafts distended to different pressure levels by using immunohistochemical methods and *in vitro* tissue baths.

**Material and methods:** Saphenous vein grafts (SVGs) of 25 patients who underwent isolated elective CABG surgery were used in this study. By using a specific mechanism, SVGs were distended to five different pressure levels for two minutes: 0 mmHg, 50 mmHg, 100 mmHg, 200 mmHg, 300 mmHg. *In vitro* tissue baths and immunohistochemical examinations were performed.

**Results:** None of the grafts distended to 300 mmHg pressure were functional in the tissue bath system. The relaxation response to carbachol of SVGs distended to 0, 50, 100 and 200 mmHg was  $97.87 \pm 4.47\%$ ,  $98.52 \pm 3.95\%$ ,  $93.78 \pm 3.64\%$ , and  $30.87 \pm 4.11\%$ , respectively. There were no statistically significant differences in terms of relaxation responses between samples distended to 0, 50, and 100 mmHg ( $p = 0.490$ ). The relaxation response of samples distended to 200 mmHg was significantly decreased ( $p = 0.021$ ). The endothelia of samples distended to 0 mmHg were almost intact in CD31 staining. Endothelial cell loss occurred at all tested distension pressures at different degrees.

**Conclusion:** *In vitro* and immunohistochemical studies revealed that distending an SVG used for coronary artery bypass grafting with pressures of 100 mmHg or less results in less endothelial damage and increases graft patency.

**Key words:** saphenous vein, bypass, endothelial injury, vasodilatation.

## Streszczenie

**Wprowadzenie:** Uszkodzenie śródbłonna spowodowane przez wysokie ciśnienia stosowane do relaksacji skurczów przy preparowaniu przeszczepu jest jedną z najbardziej prawdopodobnych teorii wyjaśniających wczesną niewydolność przeszczepu.

**Celem pracy** było zademonstrowanie uszkodzenia śródbłonna w przeszczepach żyły odpiszczelowej, na które wywierano ciśnienie o różnej wysokości przy użyciu metod immunohistochemicznych i łaźni tkankowych (*tissue bath*) przeprowadzanych *in vitro*.

**Materiał i metody:** Badano przeszczepy żył odpiszczelowych (*saphenous vein graft* – SVG) 25 pacjentów, którzy przeszli izolowane elektywne pomostowanie aortalno-wieńcowe (CABG). Przy użyciu swoistego mechanizmu oddziaływano na SVG przez 2 minuty ciśnieniem o 5 różnych wartościach: 0 mm Hg, 50 mm Hg, 100 mm Hg, 200 mm Hg oraz 300 mm Hg. Zastosowano łaźnie tkankowe *in vitro* oraz przeprowadzono badania immunohistochemiczne.

**Wyniki:** Żaden z przeszczepów, na które oddziaływało ciśnieniem 300 mm Hg, nie funkcjonował w systemie łaźni tkankowej. Odpowiedź rozkurczowa SVG, na które oddziaływało ciśnieniem 0, 50, 100 i 200 mm Hg, na karbachol wynosiła odpowiednio  $97,87 \pm 4,47\%$ ,  $98,52 \pm 3,95\%$ ,  $93,78 \pm 3,64\%$  oraz  $30,87 \pm 4,11\%$ . Nie wystąpiły żadne istotne statystycznie różnice w zakresie odpowiedzi rozkurczowej pomiędzy próbkami, na które oddziaływało ciśnieniem 0, 50 i 100 mm Hg ( $p = 0,490$ ). Odpowiedź rozkurczowa próbek, na które oddziaływało ciśnieniem 200 mm Hg, była znacząco zmniejszona ( $p = 0,021$ ). W przypadku śródbłonna próbek, na które oddziaływało ciśnieniem 0 mm Hg, barwienie CD31 ukazało niemalże nienaruszoną tkankę. Utrata komórek śródbłonna następowała przy wszystkich testowanych ciśnieniach w różnym stopniu.

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## Introduction

The saphenous vein was first used as a graft by Sabiston for coronary artery disease in 1963 [1]. Since Favaloro's interpositioning of the saphenous vein between the right coronary artery and the aorta in 1968, it has been widely used as an aortocoronary bypass conduit [2].

In the mid 1980s, two studies revealed the superior ten-year patency rates of internal thoracic artery grafts (ITA) to the saphenous vein graft (SVG) [3, 4]. Despite increased attention to arterial grafts, SVGs are still the most commonly used graft type in coronary artery bypass grafting surgery (CABG) due to their wide diameter, ease of preparation and length.

Postoperative early term occlusion rates of SVG are reported as 15-26% [5]. For late term, 10-year patency rates are reported as 50% and atherosclerotic changes were evident in the patent grafts [6]. There are many theories for failure of saphenous veins. The theory accepted by the majority of surgeons suggests that endothelia and media damage caused by high pressure for relaxation of spasm during preparation of the graft is responsible for graft occlusion [7]. There are many studies on methods for reducing endothelial damage such as keeping grafts in vasodilator agents and preparing grafts with the "no-touch" technique [8, 9]. Viaro *et al.* reported that endothelial-derived nitric oxide synthase (eNOS) levels are decreased in grafts distended with high pressure by using immunohistochemical methods [10].

## Aim of the study

We aimed to demonstrate endothelial damage in saphenous vein grafts distended to different pressure levels by using immunohistochemical methods. Also we aimed to detect whether the relaxation response originating from endothelia is altered by this damage *in vitro* in tissue baths.

## Material and methods

### Patient selection

After obtaining informed consent and the Ethical Committee's approval, SVGs of 25 patients [19 male, 6 female, mean age:  $59.52 \pm 9.09$  (44-75 years)] who underwent isolated elective CABG surgery at our institution between May 2013 and October 2013 were used in this study. By using a specific mechanism, SVGs were distended to five different pressure levels for two minutes: 0 mmHg, 50 mmHg, 100 mmHg, 200 mmHg, 300 mmHg. Eighty SVGs (16 different SVG samples of each pressure group) were examined

**Wnioski:** Na podstawie badań *in vitro* i immunohistochemicznych stwierdzono, że oddziaływanie na SVG, które używane są do pomostowania aortalno-wieńcowego, ciśnieniem 100 mm Hg lub niższym skutkuje mniejszym uszkodzeniem tkanki i zwiększa drożność przeszczepu.

**Słowa kluczowe:** żyła odpiszczelowa, pomostowanie, uszkodzenie śródbłonna, wazodylatacja.

*in vitro* in tissue baths. Afterwards immunohistochemical examination was performed in the pathology laboratory.

Patients with a history of deep vein thrombosis, venous insufficiency, venotonic drug usage, peripheral arterial disease or diabetes mellitus and patients with macroscopic varicosities were excluded from this study.

### Graft harvesting and experimental design

All grafts were harvested by the same surgeon using routine complete skin incision with scissors. The distal end of the vein was cannulated at ankle level, all branches were ligated and the graft was prepared without a pedicle. No vasodilator agent was used while harvesting grafts. After harvesting approximately 10 cm of graft, the proximal end was occluded with a vascular clamp. The SVG was distended with previously prepared heparinized saline solution (5000 units of unfractionated heparin was diluted in 1000 ml of 0.9% NaCl solution) with the help of a pressure infusion cuff with a sphygmomanometer (ERKA D-83646, Berlin, Germany) for 2 minutes. Afterwards, 2-3 cm of graft was prepared for the tissue bath system and a 1 cm segment was resected for pathologic examination. Clamped parts of the vein were not used for the study.

### Tissue bath system

The saphenous vein segments were transferred to the vascular laboratory in 4°C Krebs solution (composition: 122 mmol/L sodium chloride [NaCl], 5 mmol/L potassium chloride [KCl], 1.25 mmol/L calcium chloride [CaCl<sub>2</sub>], 25 mmol/L sodium hydrogen carbonate [NaHCO<sub>3</sub>], 1.2 mmol/L magnesium sulfate [MgSO<sub>4</sub>], 1.0 mmol/L monopotassium phosphate [KH<sub>2</sub>PO<sub>4</sub>], and 11.5 mmol/L glucose) that was continuously aerated with 95% oxygen (O<sub>2</sub>) and 5% carbon dioxide (CO<sub>2</sub>). Each graft was sliced into rings of 3 mm width. The vascular rings were suspended in the classical tissue bath system via steel hooks. Active tension of 1 to 4 g was applied to all of the samples. The vascular rings were suspended under this tension for a minimum of 60 minutes. The samples were kept alive by 37°C oxygenated Krebs solution baths every 20 minutes. In order to measure the relaxation response, the samples were exposed to phenylephrine (Sigma) (10<sup>-6</sup> M) first for submaximal constriction. Afterwards, carbachol (Sigma-Aldrich®) was used to induce nitric oxide (NO)-mediated vasodilatation. While the phenylephrine was still in the environment, carbachol was administered to the tissue bath every two minutes starting at a concentration of 10<sup>-8</sup> M and increasing in logarithmic increments to a concentration of 10<sup>-4</sup> M. The vasodilatation

response curves were obtained and recorded as described above. The data were transferred to the computer with the help of the Transducer Acquisition System (MAY IOBS 99, FDT 05 Ankara-Turkey) and stored with the MAY-MASTER MP36 analysis program.

### **Immunohistochemical evaluation**

SVG segments were fixed in 10% formaldehyde for 12 hours and than paraffin-embedded. Three cross-sectional slices were prepared from different parts of each SVG segment. The first was stained with hematoxylin and eosin and the second with CD31 antibody. CD31-immunostaining was performed to evaluate saphenous vein endothelial cells, as CD31 is a specific marker of endothelial cells, as described by Stigler et al. [11]. Antigen retrieval was performed for 30 min at 98°C. CD31 antibody (Thermo Scientific™ CD31/PECAM-1, Rabbit Polyclonal Antibody) was added for 60 min. Staining and antibody detection were performed according to the kit protocol (Thermo Scientific). Microscopically, CD31+ endothelial cells on the intimal surface were evaluated. The ratio of CD31+ endothelial cell surface to the total intimal surface of the cross-section (CD31+ endothelial cell surface and de-endothelialized tunica intima) demonstrated the percentage of endothelial cell coverage. The percentage of endothelial cell loss was noted. Endothelial cell loss results for a distinct distension pressure are expressed as the median value of three measurements in different parts of the SVG segment. Evaluation of immunohistochemistry was performed by an experienced pathologist (without prior knowledge of the features of slides).

### **Statistical analysis**

The program GraphPad Prism 6 Version Demo was used for analyzing tissue bath data. Concentration-response graphs were obtained by using statistical properties of the same program. Non-linear regression analysis (variable slope) and one-way ANOVA analysis were preferred for graphics. Intra-group analyses of groups were performed by using the *t* test.

Standard deviation, median and minimum-maximum values were used for descriptive statistics of immunohistochemical data. Kolmogorov Smirnov test was used for distribution of variables. Paired samples *t* test was used for quantified data. Data were analyzed using the Statistical Package for Social Sciences 21.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The results were assessed within a 95% confidence interval and at a level of  $p < 0.05$  significance.

## **Results**

Between May 2013 and October 2013, 25 patients were included in this study.

### **Tissue bath system**

Phenylephrine ( $10^{-6}$  M concentration) was routinely administered to grafts for achieving sub-maximal contrac-

tion. Afterwards, carbachol was administered in a cumulatively increasing manner from  $10^{-8}$  to  $10^{-4}$  M concentration and nitric oxide mediated dose-relaxation graphics were obtained separately (Fig. 1).

None of the grafts distended to 300 mmHg pressure were functional in the tissue bath system. Statistical analysis was not possible because neither a contraction nor a relaxation response was achieved.

Relaxation responses to carbachol of SVGs distended to 0, 50, 100 and 200 mmHg were  $97.87 \pm 4.47\%$ ,  $98.52 \pm 3.95\%$ ,  $93.78 \pm 3.64\%$  and  $30.87 \pm 4.11\%$ , respectively. Log EC50 values were determined as  $-5.932$ ,  $-6.016$ ,  $-6.132$ ,  $-7.434$ , respectively. There were no statistically significant differences for relaxation responses of samples distended to 0, 50 and 100 mmHg ( $p = 0.490$ ). Relaxation responses of samples distended to 200 mmHg were significantly decreased ( $p = 0.021$ ) (Fig. 2).

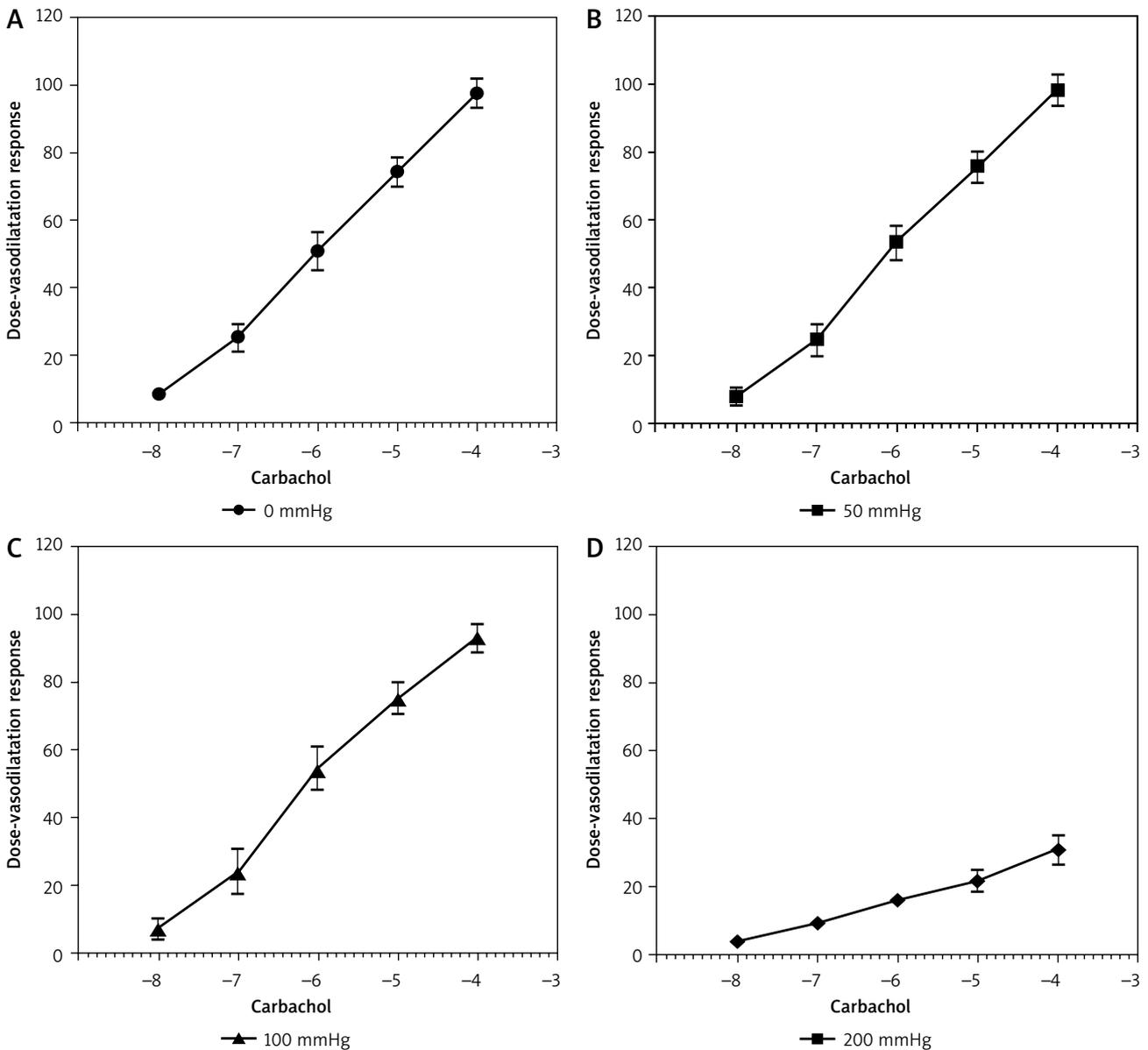
### **Endothelial cell loss of SVGs via CD31 immunostaining**

Diameters of SVGs distended to 200 and 300 mmHg were significantly increased in macroscopic evaluation (Fig. 3). Endothelia of samples distended to 0 mmHg were almost intact with CD31 staining. De-endothelialized areas were similar in groups distended to 50 and 100 mmHg. In 200 mmHg group these areas were significantly increased. There was a near total loss of endothelial cells in the group distended to 300 mmHg (Fig. 4). Endothelial cell loss occurred at all tested distension pressures at different degrees. The extent of acute endothelial cell loss increased with increasing pressure magnitude. At 0 mmHg the mean endothelial cell loss was  $0.8 \pm 1.6\%$  (range: 0-5%,  $p = \text{NS}$  or baseline), at 50 mmHg  $34.5 \pm 10.8\%$  (range: 20-55%,  $p < 0.001$ ), at 100 mmHg  $50.0 \pm 7.1\%$  (range: 40-60%,  $p < 0.001$ ), at 200 mmHg  $75.5 \pm 5.2\%$  (range: 70-85%,  $p < 0.001$ ) and at 300 mmHg  $85.0 \pm 6.7\%$  (range: 75-95%,  $p < 0.001$ ) (Table 1). The comparison of endothelial loss between each different distension pressure and baseline is shown in Figure 5.

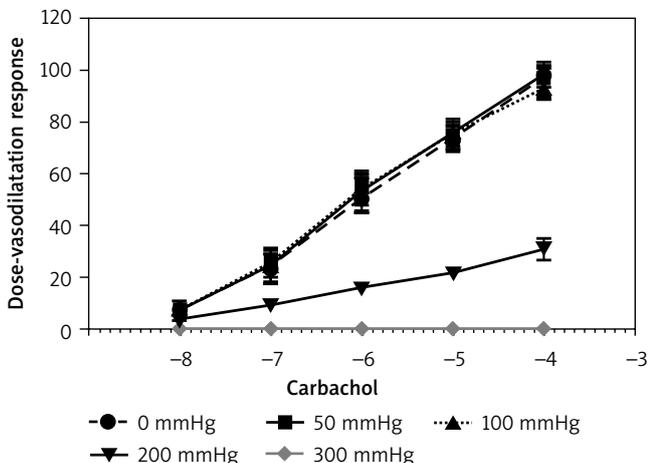
## **Discussion**

The saphenous vein is examined in three different layers: intima, media and adventitia. The intima is the luminal layer and it is covered with endothelia. Fenestrated basal membrane and intimal cells are adjacent to endothelial cells. The media layer is formed by longitudinal muscular cells connected with collagen and elastic fibers internally and circular muscular cells externally. The adventitia is the outermost layer of the vein wall.

In 1980, Furchgott and Zawadzki indicated that endothelium requires endothelium-derived relaxation factor (EDRF) for responding to acetylcholine; later on EDRF was renamed as nitric oxide (NO) [12]. Lately it was understood that endothelium is not just a barrier for blocking extravasation of blood cells and elements, but is also an organ with various biological functions. Endothelium takes part in regulating vascular smooth muscular cell tonus and hemostasis. Under normal circumstances, platelet activation, ad-



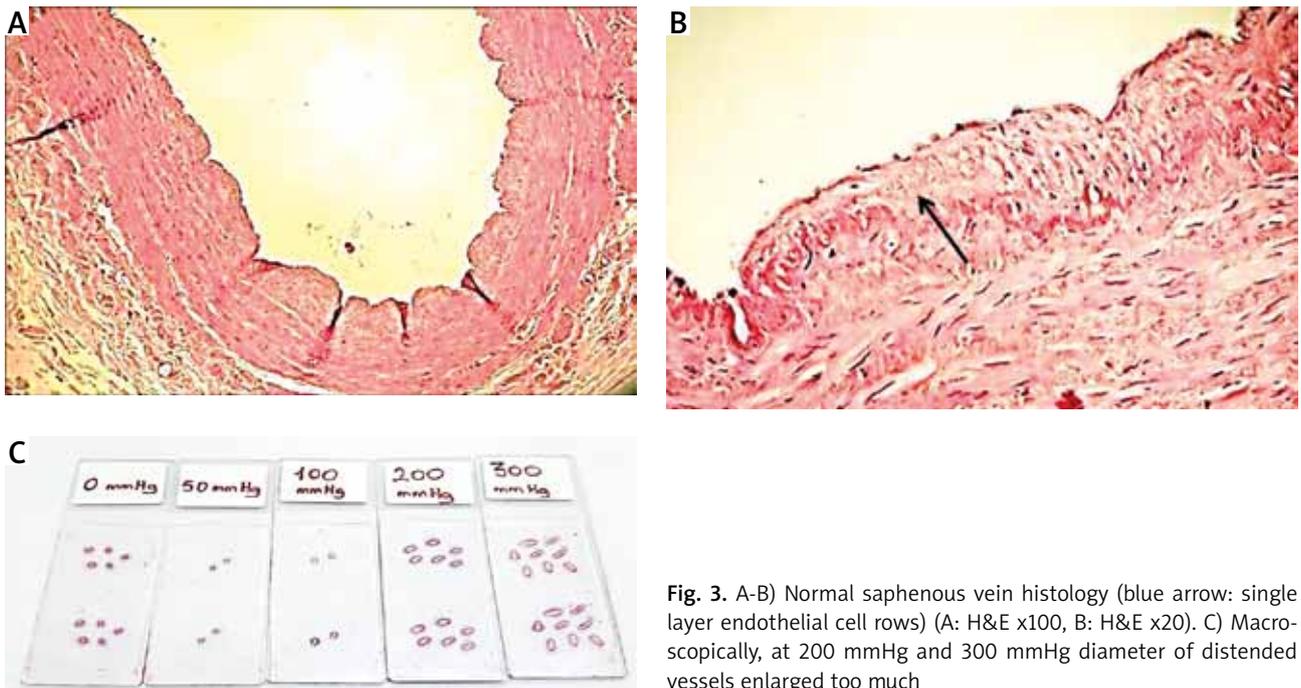
**Fig. 1.** Dose-vasodilatation response curves of SVGs. A) Dose-vasodilatation responses of 0 mmHg distended SVGs, B) Dose-vasodilatation responses of 50 mmHg distended SVGs, C) Dose-vasodilatation responses of 100 mmHg distended SVGs, D) Dose-vasodilatation responses of 200 mmHg distended SVGs



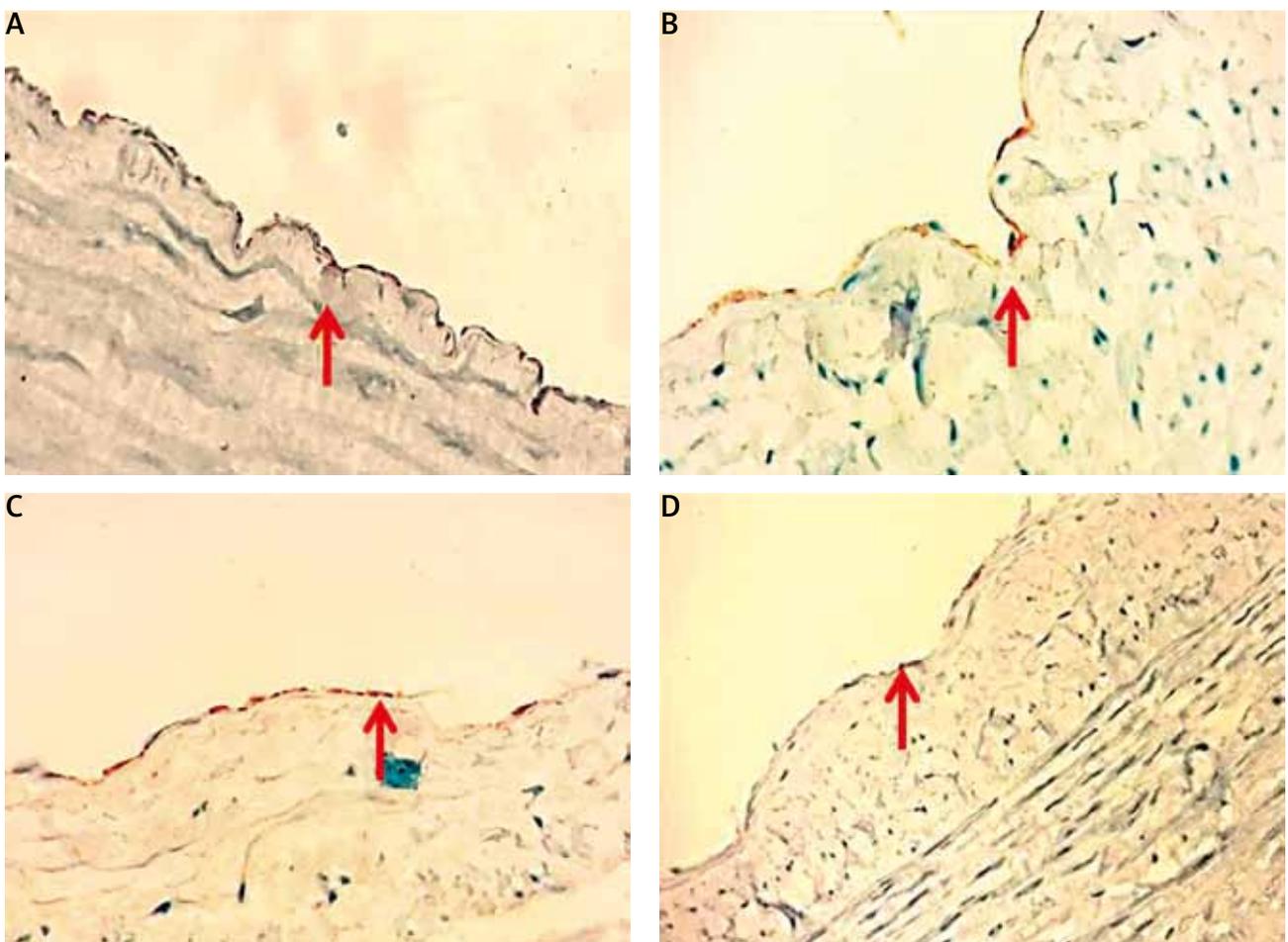
**Fig. 2.** Comparison of vasodilatation responses between different pressures

hesion and aggregation are inhibited by prostacyclin (PGI<sub>2</sub>) and EDRF that are continuously secreted from endothelium [13]. Endothelial cells also secrete antithrombin, heparin-like substances and plasminogen activator that provide local thrombolysis [14]. Endothelial integrity of the saphenous vein graft is very important not only for its physical barrier effect but also for its ability to secrete bioactive products.

Traumatic preparation of the saphenous vein graft is considered to be responsible for early graft occlusion. High pressure applied for resolving spasm in harvesting of the graft results in endothelial detachment and media damage [15]. Lipid uptake of the vein wall is considered to be responsible for late-term patency [16]. Endothelial damage after intraluminal pressures over 150 mmHg is blamed for reduced patency rates in many studies [17]. Galea *et al.* observed that apoptosis was increased in the saphenous vein



**Fig. 3.** A-B) Normal saphenous vein histology (blue arrow: single layer endothelial cell rows) (A: H&E x100, B: H&E x20). C) Macroscopically, at 200 mmHg and 300 mmHg diameter of distended vessels enlarged too much

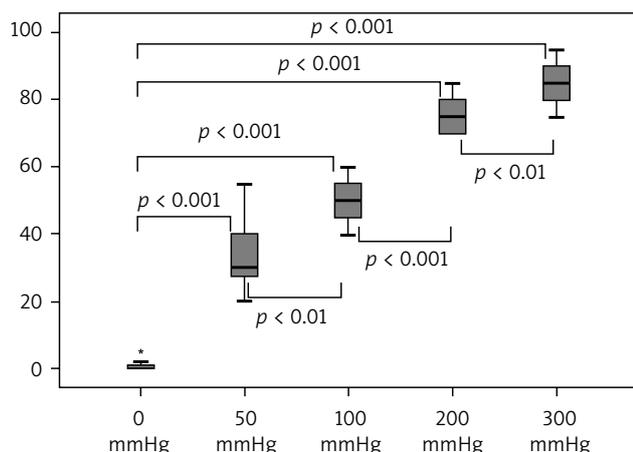


**Fig. 4.** Immunohistochemical CD31 staining examples of saphenous vein grafts. CD31 positive intimal endothelial cells stained in brown (red rows). A) In the baseline group (0 mmHg) saphenous veins show an almost completely intact intimal surface (x20). B) In 50 and 100 mmHg distension pressure groups, similarly, small de-endothelialized areas were seen (x40). C) In the 200 mmHg distension pressure group, small de-endothelialized areas are more widespread (x20). D) A distension pressure of 300 mmHg causes complete endothelial cell loss. Intimal surface is flattened (blue arrow) (x20)

**Tab. I.** Endothelial loss and the comparison of groups

	Mean ± SD	Med (Min.-Max.)	Change compared to 0 mmHg Mean ± SD	p	Change compared to pressure one below mean ± SD	p
0 mmHg	0.8 ± 1.6	0 0-5				
50 mmHg	34.5 ± 10.8	30 20-55	33.7 ± 10.0	0.000		
100 mmHg	50.0 ± 7.1	50 40-60	49.2 ± 6.8	0.000	15.5 ± 12.1	0.002
200 mmHg	75.5 ± 5.2	75 70-85	74.6 ± 5.6	0.000	25.5 ± 10.1	0.000
300 mmHg	85.0 ± 6.7	85 75-95	84.2 ± 6.7	0.000	9.5 ± 8.2	0.003

Paired samples t-test



**Fig. 5.** Box plot diagrams of endothelial cell loss due to different distension pressures (0, 50, 100, 200 and 300 mmHg). Comparison of endothelial loss between each different distended pressure and with the baseline (0 mmHg)

after distension with 350 mmHg for two minutes [18]. Viaro *et al.* found that endothelial-derived nitric oxide synthase (eNOS) levels remained unchanged in SVGs distended with 100 and 200 mmHg but were significantly lowered in SVGs distended with 300 mmHg [10]. Similar results were obtained in studies of Chester *et al.* in two minutes of distension of SVGs and Dashwood *et al.* in one minute of distension of SVGs [19, 20].

Stigler *et al.* obtained similar results in their study. They distended SVGs to 50, 100, 150 and 300 mmHg pressure for 30 minutes. They found that by using CD-31 immunostaining at 50, 100 and 300 mmHg pressure, endothelial loss levels were 29%, 54% and 91%, respectively [11].

We examined both functional and pathological changes in our study. We applied different pressures to harvested saphenous veins for two minutes and obtained similar results. Although intimal tears and endothelial loss were less in grafts distended to 0, 50 and 100 mmHg, they were noticeably higher in grafts distended to 200 and 300 mmHg. Also we found that, as correlated with these pathologic findings, endothelial-derived relaxation responses were reduced in the tissue bath at grafts distended to 200 mmHg. We believe that this is caused by reduced nitric oxide activity as a result of endothelial damage due to pressure. In grafts distended with 300 mmHg, there was neither

a contraction nor a relaxation response, which we believe was the result of damage not only at the endothelium but also at the media layer. We consider that pressures of 300 mmHg or more turn the SVG into a dead pipe. There is abundant evidence suggesting that nitric oxide levels are reduced in damaged veins. Early graft vasospasm and thrombotic occlusion may be due to reduced endothelial NO levels. NO also has effects on inhibiting platelet and leukocyte adhesion, vascular smooth muscle proliferation and migration and other antioxidant effects [21].

Irrigation solution and its temperature are also effective in endothelial protection as distension pressure and time are. We preferred heparinized isotonic 0.9% NaCl solution at room temperature for the irrigation solution. Bush *et al.* reported that the best protection is achieved at normal room temperature and 37°C. Temperature at 4°C causes separation at the basal membrane and spherical changes in cells [17]. There are many reports on different irrigation solutions. In report comparing blood and isotonic 0.9% NaCl solution, vascular contraction and endothelial loss were greater in the group kept in blood. Vascular relaxation was better in the group kept in isotonic 0.9% NaCl solution [22]. Despite reports showing that Ringer’s solution or balanced electrolyte solution does less damage than isotonic 0.9% NaCl solution, the least damage is achieved by heparinized blood [17, 23, 24]. We preferred isotonic 0.9% NaCl solution because of the required high blood amount. As we planned to investigate the relaxation response in the tissue bath, no vasodilator agent was used before the procedure.

SVG prepared with the “no touch” technique (harvesting of graft with surrounding tissue) does not require distension. The saphenous vein is not handled neither at harvesting or while performing anastomosis and reported early patency rates are 95.4% [25]. In our previous study, we also found that relaxation responses of SVGs harvested with the “no-touch” technique are better [9].

The main limitations of this study are that although use of a pressure infusion cuff with sphygmomanometer was preferred for distension, this pressure was not screened and blood was not used as a distension solution.

**Conclusions**

Examination with CD31 immunostaining revealed that endothelial damage was evident at all distension pres-

tures, but this damage was more obvious and generalized at pressures at 100 mmHg and over. Histologic and physiologic deformities were less in saphenous veins distended with 100 mmHg or less but endothelial relaxation responses were obviously decreased at 200 mmHg pressure. Especially in SVGs distended with 300 mmHg, although with endothelial and structural changes, neither vasoconstriction nor vasodilatation responses were achieved.

In conclusion, we report that distension of the SVG for coronary artery bypass grafting surgery with pressures of 100 mmHg or less will result in less endothelial damage and increased graft patency.

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### Disclosure

The authors report no conflicts of interest.

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