Endothelium-dependent and endothelium-independent flow reserve in vascular regions supplied by the internal mammary artery before and after bypass grafting

Andreas Hartmann a,*, Wolfgang Reuss b, Wolfram Burger a, Georg-Dieter Kneissl a, Wolfgang Rothe a, Friedhelm Beyersdorf c

a Department of Interventional Cardiology, St. Georg Medical Center, Delitzscher Str. 141, 04129 Leipzig, Germany
b Department of Cardiology and Department of Cardiothoracic Surgery, J.W. Goethe-University Medical Center, Frankfurt, Germany
b Department of Cardiothoracic Surgery, University Medical Center, Freiburg, Germany

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Abstract

Objective: It was the goal of this study to compare endothelium-dependent and endothelium-independent flow reserve in vascular regions supplied by the left internal mammary artery before and after bypass graft surgery. Methods: The native internal mammary artery in situ was investigated in 13 patients (age 61.8 ± 8.0 years) with angiographically proven coronary artery disease. The internal mammary artery after bypass grafting was investigated in ten patients (age 60.8 ± 7.3 years) 3.5 ± 2.8 years after the operation. Flow reserve was evaluated endothelium-dependent with acetylcholine (ACh 25 and 50 μg i.c.) and endothelium-independent with nitroglycerin (NTG 0.3 mg i.c.) followed by papaverin (10 mg i.c.). Flow indices were calculated from intraluminal Doppler blood flow velocity measurements and the vascular cross-sectional area as determined by quantitative angiography. An index for vascular resistance was defined as the ratio of pressure gradient and resting or peak flow. Results: After endothelium-dependent stimulation with acetylcholine 25 μg (50 μg), flow in the internal mammary increased by 352.3 ± 152% (412 ± 145%) before surgery, whereas it increased only by 213 ± 134% (193 ± 120%) after surgery (P < 0.05). Endothelium independent stimulation with papaverin resulted in a flow increase of 391 ± 234% before surgery vs. 315 ± 135% after surgery (n.s.). The resistance index decreased after endothelium-dependent stimulation with acetylcholine 25 μg(50 μg) to 35 ± 16.8% (28 ± 8.9%) before surgery, whereas it decreased only to 59 ± 26% (72 ± 43%) after surgery (P < 0.05). Endothelium independent stimulation with papaverin resulted in a decrease of the vascular resistance index to 31 ± 14% before surgery vs. 32 ± 14% after surgery (n.s.). Conclusion: Vascular regions supplied by the internal mammary artery as a graft demonstrate a significantly reduced endothelium-dependent flow reserve but a preserved endothelium-independent flow reserve as compared to vascular regions supplied by the native internal mammary artery. The selective decrease in endothelium-dependent flow reserve may be due to microvascular changes in the myocardial region supplied by the internal mammary artery after bypass grafting. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Endothelium; Coronary artery bypass graft; Coronary flow reserve; Microcirculation

1. Introduction

Aortocoronary bypass surgery utilizing the internal mammary artery as a bypass graft was introduced in 1968 [1]. Follow-up investigations up to 20 years after the initial operation have demonstrated superior patency rates and improved clinical results with internal mammary artery graft surgery as compared to vein graft surgery [2,3]. It was found that the use of the internal mammary artery as a bypass graft is a more important predictor of survival than the progression of native coronary atherosclerosis [3].
The superiority of the internal mammary bypass graft over the saphenous vein graft has been attributed to the favourable biological properties of the endothelium protecting the arterial graft against vasospasm, thrombus formation and atherosclerosis [4]. Several studies have addressed the issue of physiological changes in the native internal mammary artery after bypass surgery. It has been demonstrated in vivo, that endothelial function in the internal mammary is preserved before and after bypass surgery [5]. This observation was made even in the presence of severe coronary artery disease where endothelial function is impaired in early stages of the disease [5,6]. Physiological changes of flow patterns have been described in the internal mammary artery after bypass surgery with redistribution of blood flow velocity from systole to diastole [7]. It has been hypothesized that blood flow velocity and its dynamic pattern have the potential to modulate endothelial responses with important implications for vessel wall homeostasis and bypass conduit function and longevity [8]. However, physiological changes of endothelium-dependent in comparison to endothelium-independent flow reserves in different vascular regions supplied by the internal mammary artery before and after bypass surgery have not been evaluated.

It was the purpose of this study to investigate in vivo endothelium-dependent and endothelium-independent flow regulation in vascular regions supplied by the internal mammary artery before and in the long-term follow-up after coronary artery bypass grafting.

2. Patients and methods

2.1. Patients

A total of 13 patients with coronary artery disease (1 female, 12 male, age 61.7 ± 8.05 years) but without bypass surgery and ten patients (1 female, 9 male; age 60 ± 2.3 years) after coronary bypass surgery with internal mammary artery grafts were included in the study. The study protocol was approved by the Ethical Committee of the J.W. Goethe-University, Frankfurt/Main. All patients gave informed consent to the investigation being performed during the routine cardiac catheterization for evaluation of coronary artery disease. All internal mammary artery grafts were anastomosed to the left anterior descending coronary artery. Mean age of the arterial grafts was 3.5 ± 1 years. Five patients in the group with internal mammary artery grafts had electrocardiographical signs of myocardial scarring in the bypass-perfused region. All bypassed coronary arteries were completely occluded with blood supply to the anastomosed vascular regions exclusively via the graft.

2.2. Study protocol

Left heart catheterization was carried out in both groups by a standard femoral approach. After termination of the diagnostic procedure, a 3-F Millar DC-201 Micro Tip 20 MHz catheter (Millar Instruments, Houston, Texas) was positioned over a PTC guidewire through an 8-F guiding catheter in the mid segment of the left internal mammary artery. Velocity signal generation and processing were achieved using a standard velocimeter (Model MDV-20, Millar) range-gated and calibrated for use with the 3-F (DC-201 Millar) Doppler catheter with an internally set calibration of 0–100 cm/s (1 KHz = 3.75 cm/s) for full-scale deflection [9]. The velocimeter was connected to a multichannel oscillographic monitor and strip chart recorder (Cardiognost, Hellige, Freiburg, Germany). During baseline conditions, the position of the Doppler flow-velocity catheter and the range gate control were adjusted to optimize the audio flow-velocity signal and the phasic flow-velocity waveform. The Doppler catheter position and the range gate control were not changed thereafter. Intraarterial velocity and arterial pressures were recorded continuously at baseline and during the administration of the study agents.

Angiography was performed with a monoplane or a biplane imaging system set up in a right and left oblique position with adequate cranial or caudal angulation allowing analysis of the internal mammary artery as well as the tip of the intraluminal Doppler catheter on end-diastolic frames. Special care was taken not to let the guiding catheter tip wedge into the vessel origin. Position of the imaging tube was kept constant during the protocol. Angiograms were obtained by injection of 10 ml of a nonionic contrast agent (Ultravist, Schering AG, Berlin, Germany). Angiographic dimensions were assessed by a validated quantitative angiography analysis system (Medis, Leiden, The Netherlands).

The study agents were injected into the internal mammary artery through the 3-F intraluminal Doppler catheter. A total of 25 μg acetylcholine chloride and 50 μg acetylcholine chloride (Dispersa, Germering, Germany) were dissolved in 10 ml of 0.9% saline and injected into the internal mammary artery with an injection rate of 0.5 ml/s as described by Yasue et al. [10]. After each injection and after registration of flow velocities, arteriography was performed. Acetylcholine was then followed by intraarterial application of 0.3 mg of nitroglycerin to achieve maximal arterial vaso- dilatation. Maximal flow was then induced by the administration of papaverin in a dose of 10 mg i.a.

2.3. Data analysis

The diameters of the internal mammary artery were measured at the location of the sample volume of the
Doppler catheter. From these measurements, the cross sectional area of the internal mammary artery 2–4 mm distal to the tip of the Doppler catheter was determined. For estimation of directional changes in blood flow, a flow index was calculated by multiplying the mean-Doppler derived blood flow velocity with the computed cross-sectional area of the internal mammary artery distal to the Doppler tip [6]. From the flow indices and the pressure gradient, a resistance index was calculated. Additionally, internal mammary artery dimensions were determined to exclude limitations of flow caused by internal mammary artery constriction in response to acetylcholine. As suggested previously, flow limitations have to be expected by >50% diameter constriction in the most constricted segment distal to the Doppler-catheter tip [11].

2.4. Statistical analysis

Responses of vessel diameters to acetylcholine or nitroglycerin were expressed as percent change of the diameter from baseline [10]. Variations of flow parameters after acetylcholine, nitroglycerin and papaverin were expressed as percent change from baseline.

Data are presented as mean ± standard deviation (S.D.). Variations of hemodynamic parameters, arterial dimensions and flow parameters within each group were evaluated by analysis of variance (ANOVA). In case of significance, the Wilcoxon Signed Ranks test was then used to compare pre-intervention data to values obtained following the application of acetylcholine, nitroglycerin and papaverin. Comparisons between two groups were performed with two-tailed t-testing. A probability level $P < 0.05$ was considered significant.

### Table 1

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>Baseline</th>
<th>Acetylcholine 25 µg</th>
<th>Acetylcholine 50 µg</th>
<th>Nitroglycerin 0.3 mg</th>
<th>Papaverin 10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>72 ± 12</td>
<td>73 ± 10</td>
<td>73 ± 10</td>
<td>74 ± 12</td>
<td>75 ± 13</td>
</tr>
<tr>
<td>Graft</td>
<td>74 ± 9</td>
<td>73 ± 9</td>
<td>72 ± 8</td>
<td>75 ± 10</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>98 ± 14</td>
<td>100 ± 12</td>
<td>102 ± 17</td>
<td>99 ± 13</td>
<td>97 ± 17</td>
</tr>
<tr>
<td>Graft</td>
<td>110 ± 12</td>
<td>108 ± 12</td>
<td>112 ± 21</td>
<td>104 ± 14</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>Internal mammary artery diameter (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>0.27 ± 0.05</td>
<td>0.3 ± 0.05</td>
<td>0.3 ± 0.05</td>
<td>0.29 ± 0.06</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>Graft</td>
<td>0.21 ± 0.03</td>
<td>0.2 ± 0.03</td>
<td>0.21 ± 0.05</td>
<td>0.22 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
</tbody>
</table>

* $P < 0.05$ vs. baseline; + $P < 0.05$ native artery vs. internal mammary artery graft.

### 3. Results

#### 3.1. Systemic hemodynamics

The effect of selective application of 25 and 50 µg acetylcholine, 0.3 mg nitroglycerin and 10 mg papaverin into the internal mammary artery resulted in minor and insignificant changes in systemic blood pressure and heart rate (Table 1).

#### 3.2. Endothelium-dependent and endothelium-independent vasomotion of the internal mammary artery before and after bypass graft surgery

Table 1 lists the effect of cumulative doses of 25 and 50 µg acetylcholine and 0.3 mg nitroglycerin on the diameters of the internal mammary artery before and after bypass graft surgery. The diameter of the native internal mammary artery changed by 8.4 ± 9.6% and by 11.2 ± 13.2% after 25 and 50 µg acetylcholine and by 7.8 ± 12.8% and 13.5 ± 16.6% after 0.3 mg nitroglycerin and 10 mg papaverin. The diameter of the internal mammary artery after bypass surgery changed by $-2.5 ± 9.8\%$ and by $-2.2 ± 13.7\%$ after 25 and 50 µg acetylcholine and by $4.0 ± 7.3\%$ and $3.5 ± 9.6\%$ after 0.3 mg nitroglycerin and 10 mg papaverin.

These changes were statistically not significant compared to baseline conditions or comparing the changes in the internal mammary artery before and after bypass surgery.

#### 3.3. Endothelium-dependent and endothelium-independent regulation of coronary blood flow in vascular regions supplied by the internal mammary artery before and after bypass grafting

Fig. 1 depicts the response of the flow index to applications of 25 and 50 µg of acetylcholine, 0.3 mg...
nitroglycerin and 10 mg papaverin into the internal mammary artery. After endothelium-dependent stimulation with 25 and 50 μg acetylcholine, flow index increased by 252 ± 146% (P < 0.05 vs. baseline and vs. internal mammary artery graft) and 312 ± 139% (P < 0.05 vs. baseline and vs. internal mammary artery graft) in vascular region supplied by the native internal mammary artery. Flow index increased after 25 and 50 μg by 113 ± 127% (P < 0.05 vs. baseline) and 93 ± 111% (P < 0.05 vs. baseline) in vascular region supplied by the internal mammary artery graft.

After endothelium-independent stimulation, flow index increased significantly in the internal mammary artery before and after surgery as compared to baseline values. Flow index increased by 191 ± 127% (P < 0.05 vs. baseline) after 0.3 mg nitroglycerin and by 291 ± 127% (P < 0.05 vs. baseline) after 10 mg papaverin in vascular region supplied by the native internal mammary artery. Flow index increased by 150 ± 107% (P < 0.05 vs. baseline) after 0.3 mg nitroglycerin and by 215 ± 128% (P < 0.05 vs. baseline) after 10 mg papaverin in vascular region supplied by the internal mammary artery graft.

Increases of flow index after endothelium-dependent stimulation with acetylcholine were significantly higher in vascular regions perfused by the native internal mammary artery as compared to the graft. After nitroglycerin, the flow increase was not significantly higher in the native internal mammary artery as compared to the internal mammary artery graft. The flow increase after 10 mg papaverin also, was not significantly different before and after graft surgery.

Fig. 2 displays the response of the resistance index to application of 25 and 50 μg of acetylcholine, 0.3 mg nitroglycerin and 10 mg papaverin. After endothelium-dependent stimulation with 25 and 50 μg acetylcholine, resistance index decreased to 65 ± 16% (P < 0.05 vs. baseline and vs. internal mammary artery graft) and to 72 ± 9% (P < 0.05 vs. baseline and vs. internal mammary artery graft) in vascular region supplied by the native internal mammary artery. Resistance index decreased to 41 ± 24% (P < 0.05 vs. baseline) and to 28 ± 39% (P < 0.05 vs. baseline) after 25 and 50 μg acetylcholine in vascular region supplied by the internal mammary artery graft.

After endothelium-independent stimulation, resistance index decreased significantly in the internal mammary artery before and after surgery as compared to baseline values. Resistance index decreased to 58 ± 18% (P < 0.05 vs. baseline) after 0.3 mg nitroglycerin and to 69 ± 13% (P < 0.05 vs. baseline) after 10 mg papaverin in vascular region supplied by the native internal mammary artery. Resistance index decreased to 56 ± 15% (P < 0.05 vs. baseline) after 0.3 mg nitroglycerin and to 68 ± 14% (P < 0.05 vs. baseline) after 10 mg papaverin in vascular region supplied by the internal mammary artery graft.

Decreases of resistance index after endothelium-dependent stimulation with acetylcholine were significantly higher in vascular regions perfused by the native internal mammary artery as compared to vascular regions supplied by the internal mammary artery graft. After nitroglycerin, the decrease of the resistance index was not significantly higher in the native internal mammary artery as compared to the internal mammary artery graft. The flow increase after 10 mg papaverin also, was not significantly different before and after graft surgery.
4. Discussion

Improved patency rates of internal mammary artery grafts as compared to saphenous vein grafts have long been established. A total of 1 year after the operation, 76–93% of vein grafts are open but at the end of 10 years only 41–63% remain patent. Patency rates for internal mammary artery grafts are 88–96% at 1 year and 69–83% at 10 years [12,13].

The internal mammary artery undergoes a profound change in physiology by being harvested as a bypass graft. Prior to surgery, the vessel supplies a microcirculatory region in skeletal muscle with a predominantly diastolic flow pattern. After being anastomosed as a bypass graft, the internal mammary artery supplies the microcirculation of the myocardium with a predominantly systolic flow pattern. Although the internal mammary artery has been shown to be superior to the native internal mammary artery as a coronary bypass graft, the internal mammary artery supplies the myocardium with a well-preserved endothelial function., the issue remains what the effects are of transferring the internal mammary artery from its native physiologic site to becoming a coronary bypass graft.

4.1. Endothelium-dependent flow regulation in vascular regions supplied by the internal mammary artery before and after bypass surgery

Endothelium-dependent and endothelium-independent coronary flow reserve and coronary resistance were evaluated in this investigation as functional parameters of the microcirculation before and in the long-term course after bypass surgery with internal mammary artery grafts.

Endothelium-dependent stimulation with acetylcholine resulted in a significant flow increase in the native internal mammary artery. The increase of the flow index was $252 \pm 146\%$ after $25 \, \mu g$ and $312 \pm 139\%$ after $50 \, \mu g$ of acetylcholine. Acetylcholine-induced endothelial stimulation has been shown in non-surgically treated subjects with normal coronary arteries and without chest pain to result in a considerable increase in coronary flow of up to 345% [14]. Compared with these findings, the endothelium-dependent flow increase after acetylcholine in the native internal mammary artery is within a normal range in this cohort of patients with coronary artery disease. After bypass grafting there was only an increase of $113 \pm 127\%$ in flow index after $25 \, \mu g$ of acetylcholine and of $93 \pm 111\%$ after $50 \, \mu g$ of acetylcholine. The difference of flow increase in the native internal mammary artery compared to the flow increase in the internal mammary artery after coronary bypass grafting was statistically significant.

Resistance, taking into consideration possible changes in blood pressure, was significantly reduced by acetylcholine. Again, the reduction in vascular resistance was significantly greater in vascular regions supplied by the native internal mammary artery as compared to vascular regions supplied by the internal mammary artery as a coronary bypass graft.

Internal mammary artery grafts have been shown to have a well preserved endothelial function, especially if compared to venous bypass grafts. In vitro studies have demonstrated an increased release of EDRF and prostacyclin from internal mammary artery as compared to saphenous vein [4,15].

Generally, it has been suggested that arterial grafts demonstrate a similar activation and behaviour of the L-arginine pathway independent of the type of arterial bypass graft [16]. Several studies utilizing different methods have observed a preserved endothelial function in the long-term follow-up after internal mammary artery bypass grafting [5,17–19]. Endothelial stimulation was performed pharmacologically with acetylcholine [5], substance P [19] or by increasing flow with pacing [18] and papaverin [17].

The change in endothelium-dependent flow reserve in the internal mammary artery after bypass surgery may be due to a chronic change in flow pattern and shear stress. Hanet et al. [7] found that implantation of the internal mammary artery to the coronary circulation resulted in redistribution of the blood flow velocity pattern towards diastole. This has been attributed to an adaptation of blood supply to myocardial metabolic requirements.

A difference in phasic blood flow patterns comparing arterial and venous grafts has been described by Bach et al. [8]. In situ internal mammary artery grafts demonstrated a gradual longitudinal transition in the phasic flow pattern from predominantly systolic velocity proximally to predominantly diastolic velocity distally. Saphenous vein graft flow velocity pattern showed a consistently diastolic predominance [8]. Mean flow velocities, total velocity integral and calculated maximal shear rates were significantly higher in all segments of internal mammary arteries compared with values in saphenous vein grafts.

It may be concluded in agreement with the findings cited above, that since phasic blood flow and shear stress have the potential to directly modulate endothelial responses [20], differences in phasic blood flow patterns may be an explanation for the better preserved endothelial function in microcirculatory regions supplied by the internal mammary artery before bypass grafting. Another explanation is that changes of endothelium-dependent flow reserve after bypass grafting are due to structural changes in the microcirculation of the myocardial region perfused by the anastomosed left anterior descending coronary artery. These changes may even be late sequelae of the surgical intervention.
4.2. Endothelium-independent flow regulation in vascular regions supplied by the internal mammary artery before and after bypass surgery

Endothelium-independent stimulation with the microcirculatroy vasodilator papaverin resulted in a 2–3-fold increase of coronary blood flow. There was a tendency towards a higher flow increase in microvascular regions supplied by native internal mammary arteries. However, the difference as compared to the flow increase in regions with internal mammary artery bypass grafts was not statistically significant. The calculated vascular resistance after papaverin was similar in vascular regions supplied by internal mammary artery grafts as compared to regions supplied by the native internal mammary artery. Although there were electrocardiographical signs of scarring in five patients with internal mammary artery grafts, endothelium-independent flow reserve was normal. Consequently, myocardial scarring in these patients was not hemodynamically relevant because extensive scarring reduces endothelium-independent coronary flow reserve.

Variations of endothelium-independent flow reserve can be observed in arterial grafts depending on the time after operation, especially in the early phase[17,21]. A normal flow reserve after endothelium-independent microcirculatory vasodilation with papaverin or dipyridamole has been reported in vascular regions supplied by arterial grafts 1 year after operation [17,21]. Therefore, although the internal mammary artery supplies two different vascular regions before and after surgery, endothelium-independent flow reserve remains unchanged.

In conclusion, vascular regions supplied by native internal mammary arteries have a better preserved microcirculatory vasodilator response to acetylcholine as compared to vascular regions supplied by internal mammary artery grafts. This indicates a better preservation of microcirculatory endothelial function. However, maximal vasodilator capacity and reduction of vascular resistance after endothelium-independent stimulation was unchanged with and without bypass graft surgery indicating intact vasodilator capacity in the microcirculation in both vascular regions.

References